DIRECT PULP CAPPING

a biological approach

M. WIJNBERGEN-BUIJEN VAN WEELDEREN

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a biological approach to replace Formocresol pulpotomy by direct pulp capping

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF DR P G A B. WIJDEVELD VOLGENS HET BESLUIT VAN HET COLLEGE VAN DEKANEN IN HET OPENBAAR TE VERDEDIGEN OP DONDERDAG 24 JUNI 1982 DES NAMIDDAGS TE 4 UUR

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To all who inspired and helped me.

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Formocresol pulpotomy after exposure of vital deciduous molars has been routinely applied by the staff of the Department of Pedodontics for years. An evaluation conducted by the Department of the effects of this treatment revealed somewhat disappointing long-term results (Wijnbergen and Burgersdijk, 1976).

During the ensuing discussion among the staff of the Department, the following questions were raised:

- Is there really a need for pulp treatment of deciduous teeth ?
- If so, is there not a simpler and better method than Formocresol pulpotomy ?
- And, if such a method could be found, what criteria should it satisfy ?

The motivation for a positive reply to the first question was based largely on the following three reasons:

1) A decision, too lightly taken, to extract deciduous teeth has repercussions on the attitude of parents and children, who are then likely to regard any other activities, intended to convince them of the importance of a caries-free deciduous dentition, with some suspicion;
2) If, over a period of some years, cavities in deciduous teeth have been treated to avoid extractions - sometimes at the expense of much time and trouble - a decision to extract a tooth after an exposure can be an extremely frustating experience for patient and parents;
3) Although studies have shown that premature extraction of deciduous molars negatively affects the space avai-

lable for premolars in only 25 per cent of the patients, (Helm, and Siersbaek-Nielsen, 1973) natural shedding of deciduous molars is preferred for orthodontic reasons.

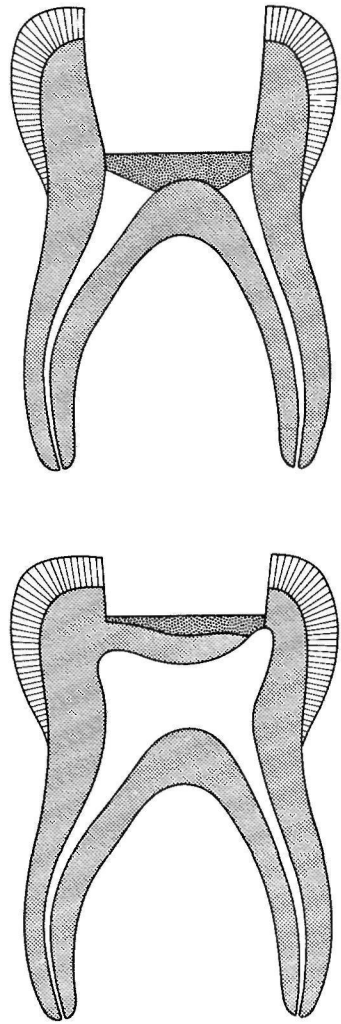
If, in the light of these arguments, it is decided not to extract an exposed deciduous tooth, the vitality of the pulp should be preserved because:

- A vital pulp will not affect the physiological process of shedding;
- A vital pulp will reduce the risk of damage to the germ of its successor. A nonvital pulp can lead to periapical or intraradicular inflammation;
- A complete mechanical removal of the nonvital content of the root canal is impossible because of the erratic pattern of root canals in deciduous molars.

Not only was the Department's evaluation of Formocresol pulpotomy somewhat disappointing, other studies are revealing that - in contrast to the generally held belief - the root pulp does not remain vital after Formocresol pulpotomy. The Department therefore decided to begin a search for a more biological method of treating exposed pulps, in collaboration with the Department of Oral Histology.

This thesis presents the first results of that study.

Fig. Diagrammatic representation of the situation after pulpotomy and application of the base (top) and after exposure and pulp capping (bottom).
Base and capping material: heavily dotted.



1. SOME HISTORICAL REMARKS ON THE TREATMENT OF EXPOSED PULPS

Summarized in this chapter is the research found in the literature on ways of treating exposed pulps in an attempt to preserve the vitality of the pulp. Although most of these studies are dealing with the pulp of permanent teeth, some of them are directed specifically to primary teeth. The reported treatments in the latter studies are:

a) pulpotomy
b) pulp capping

Both treatments are still widely practised and both have their strong adherents.

1.1 Pulpotomy

Seltzer and Bender (1975) define pulpotomy as "the removal of the coronal portion of the pulp and covering the remaining pulp stump with a medicated dressing in order to maintain the vitality of the radicular pulp tissue".
The most frequently used dressings contain calcium hydroxide or Formocresol. These and some other dressings are discussed in the following sections.

1.1.1 Pulpotomy with a paste of calcium hydroxide and water

From the biological point of view, calcium hydroxide seems to be a suitable dressing after a pulpotomy since it can promote wound healing by hard tissue bridge formation (Glass and Zander, 1949). To affirm the widely divergent outcome of experiments of capping with calcium hydroxide after pulpotomy, the results of two investigations will be described.

Schröder (1978), reported only 59 per cent successfull treatments after two years follow up, using clinical and radiographic criteria.
She stated that the most probable causes of failure were: the presence of an inflammation of the pulp tissue at the moment of treatment or the presence of a blood clot between the pulp wound and the calcium hydroxide dressing after pulpotomy.

The formation of a blood clot can be observed during treatment, but if the absence of inflammation is a prerequisite for treatment success, the problem arises how to make a correct clinical diagnosis of the state of the pulp at the moment of exposure. This had appeared to be virtually impossible, because a poor correlation exists between clinical and histological findings (Magnusson, 1970; Schröder, 1978). However, Koch and Nyborg (1970) stated that, on the basis of detailed history and examination, a correlation of 87 per cent exists between clinical and histological indications for pulpotomy.

In a recent investigation of covering pulpotomized dog pulps with calcium hydroxide in powder or paste form, after 30 days almost 90 per cent of the teeth showed a total hard tissue bridge protecting a vital and non-inflamed pulp (Holland, de Mello, Mery, et al.,1981).

1.1.2 Other drugs used after pulpotomy

Dressings, such as zinc oxide eugenol (Glass and Zander, 1949; Berger, 1965) gave low success scores after pulpotomy, internal resorption occurring frequently.
Applications of corticosteroids may offer good results clinically by temporary relieving pain, but inflammation of the pulp and resorption of the adjacent dentine remained

(Hansen, Ravn and Ulrich, 1971; Langeland et al., 1971). Mixtures of corticosteroids with antibiotics (Kiryati, 1958), antibiotics with barium sulfate (Via, 1955), calcium hydroxide with antiseptics (Citron, 1977; Marti, Thomas and Fuller, 1981) were all tried as dressings with varied success rates, each with their own advantages and disadvantages.

1.1.3 Pulpotomy with Formocresol[R]

Better known a "The" Formocresol pulpotomy. This most commonly used therapy specially for exposed pulps of primary teeth is a 5-minute application of Formocresol followed by a dressing of zinc oxide eugenol containing Formocresol. Formocresol was introduced by Buckley (1904). It consists of 19 per cent formaldehyde and 35 per cent cresol in a vehicle of 15 per cent glycerine in water.

Sweet (1930) was mainly responsible for popularizing the use of the Formocresol pulpotomy in cariously exposed primary teeth. According to Berger (1965) the formaldehyde in Formocresol causes necrotic changes in pulpal tissue which originally was vital. Rölling, Hasselgren and Tronstad (1976), Rölling and Lambjerg-Hansen (1978), Magnusson (1978) and Mejåre and Larsson (1979), described a great variety of tissue reactions in human pulp, mostly chronic inflammation and devitalization in various degrees of the root pulp and/or the periapical periodontium.

Formocresol[R] is toxic (Ranly and Fulton, 1976; Massler and Mansukhani, 1959) and in full strength it depresses the respiratory activities of fibroblasts, the RNA synthesis, and connective tissue matrix production (Loos, Straffon and Han, 1973).

Magnusson (1978) could see no vital remnants in the apical

part of the treated roots, nor any sign of healing. The extent of the devitalization of the pulp depends on the ability of the components of Formocresol^{R} diasipate from the dressing (Mejàre and Larson, 1979), the exposure time of the tissue to Formocresol^{R} (Beaver, Kopel and Sabes, 1966) and the dilution of Formocresol^{R} (Loos and Han, 1971; Morawa, Straffon, Han et al., 1975).

*Another noxious effect of Formocresol^{R} is that it com*promises the micro-circulation of the dental pulp (Langeland, 1971), formaldehyde being the main factor behind the vascular changes (Simon and van Mullem, 1978).
As a vascular connection exists between the primary tooth and the developing permanent tooth bud, it is not surprising that clinical studies indicate a relationship between Formocresol pulpotomy in primary teeth and enamel defects in their successors (Pruhs, Olen and Sharma, 1977; Burgersdijk, Jeurissen and Schols, 1982). Other investigators, however, did not find any such relationship (Rølling and Poulsen, 1978).

The clinical survival rate of 78 per cent after two years Formocresol pulpotomy (Rølling and Thylstrup, 1975; Wijnbergen and Burgersdijk, 1976) - so promising in comparison with the 59 per cent of success after pulpotomy and dressing with calcium hydroxide (Schröder, 1976) - is not in agreement with the histological findings. In vivo experiments outside the oral area demonstrated that formaldehyde-fixed autologous and bacterially non-contaminated tissue evoked chronic inflammatory reactions (Makkes, Thoden van Velzen and van den Hooff, 1978). Moreover the conclusion that Formocresol pulpotomy can result in total or almost total devitalization of the root pulp accompanied by complex vascular changes (Mejàre, 1979; Simon and van Mullem, 1978) makes it interesting to search for a more

biological therapy.

The Formocresol pulpotomy cannot fulfil its aim i.e.: to preserve vitality of the radicular pulp tissue after removal of the coronal portion.

1.2 Pulp capping

The other way of treating an exposed tooth is direct pulp capping. This is a procedure in which an attempt is made to maintain the vitality of the entire pulp that has been exposed accidentally or in the course of removing carious dentine. To this end the exposed pulpal tissue, including the tissue present in the pulp chamber, is covered with a capping material.

1.2.1 Direct consequences of a pulp exposure

Firstly exposure of a pulp means making a pulp wound. This is an infliction whether to a healthy pulp or to a pulp which was already partially inflamed. The defense reaction of the body to a wound is an inflammation, and in the case of an existing inflammation, the reaction to the wounding is cumulative.

Although in principle inflammatory changes in the pulp are the same as those elsewhere in the body, they may be modified by the anatomic confines of the pulp chamber. Pressure may be building up because of the edema and can cause pain. Venous constriction may also be provoked via edema and can cause vascular strangulation. Thus, the pulp succumbs to the pressure of the inflammatory exudate (Woehrlen, 1978).

Secondly, exposure of a pulp can cause bacterial contamination. The bacteria can originate from the carious den-

tine, from non-sterile instruments or from the saliva (Künzel, 1968; Phaneuf and Patterson, 1976; Frankl and Ruben, 1968; Baume and Holz, 1981). If these micro-organisms are not killed during subsequent treatment they can also provoke an inflammatory reaction or contribute to it (Brännström and Nyborg, 1973). The vitality of the pulp might therefore be favoured if the inflammation could be controlled in the early stages after exposure (Löringzy - Landgraf, 1956).

1.2.2 What are the criteria for a successful capping ?

In the past one was content with merely a lack of clinical symptoms after capping, but although teeth can remain vital and free of clinical symptoms, extensive inflammation and internal resorption may occur (Langeland, Dowden, Tronstad, et al., 1971). For Tronstad and Mjör (1972) and Woehrlen (1978) an acceptable goal is a pulp free of symptoms, but not completely walled off by hard tissue. Glass and Zander (1949) stated that "healing may be defined as the restoration of a tissue to its normal structure and function". As the dental pulp is normally encapsulated by dentine and comprises an adherent odontoblastic layer, their definition of the criteria for successful capping means that not only the presence of healthy tissue is required, but also a continuous odontoblastic layer and the establishment of a dentine barrier, walling off the exposure.

Maintaining the vitality of the pulp might seem an unpretentious goal and a pulp free of inflammatory changes a more sophisticated one, but healing of the wound surface, in the sense of hard tissue bridge formation is a questionable necessity.

1.2.3 Is healing possible ?

It is generally agreed that the dental pulp of human and animal teeth has the ability to repair spontaneously following carious and traumatic exposure (Kopel, 1976; Massler, 1972; Torneck, 1972; Naume and Holz, 1981).

As a reaction to an exposure, fibroblasts from the pulp-tissue elaborate a matrix that undergoes mineralization. Some cements are said to have a great ability to "irritate" the pulp to hard-tissue formation (Phaneuf, Frankl and Ruben, 1968; Stanley and Lundy, 1972; Tronstad, 1974). This process does not always proceed to completion, i.e. to a complete bridge of hard tissue over the wound (Seltzer and Bender, 1975).

Especially in cases where a carious exposure has occurred, the chance of healing is very low (Langeland et al., 1971). However, Berk and Krakow (1972) stated that depending on critical inspection of the condition of the pulp by subjective and radiographic evaluation, even carious exposures can be successfully capped.

The results of an investigation to study pulp reaction to capping materials, using deciduous and permanent monkey teeth, supported the premise that the pulp has a great capacity to survive surgical trauma and to undergo repair. Also of interest in this investigation was the statement that no apparent differences were found in the pulp reaction of deciduous and permanent teeth (Weiss and Bjorvatn, 1970), this in contrast to statements by Kopel (1976).

There are also different opinions on whether the age of an adult patient and the size of the exposure are of consequence for the healing powers of the dental pulp (Berk, 1963; Künzel, 1968).

The effects of the following factors on the healing of

the wounded pulp tissue are reported:

1. Bacterial contamination (Patterson, 1976, Woehrlen, 1978);
2. The use of damaging drugs (Seltzer, Bender and Kaufman, 1961; Stanley, Going and Chauncey, 1975);
3. The composition of capping material;
4. Dentin debris (Seelig, Fowler and Tanchester, 1954; Kalnins and Frisbie, 1960);
5. The exposure site (Horsted, El Attar and Langeland, 1981);
6. The lack of a proper peripheral seal (Brännström and Nyborg, 1973).

Conclusion: healing is possible although not in the literal sense but in the sense of regaining a condition of healthiness without inflammatory reaction and perhaps without the same cellular composition and enhousing as before the trauma.

1.2.4 The capping materials

As the aim of pulp capping is to maintain the vitality of the pulp tissue, the choice of capping materal is of crucial importance to the outcome of the treatment. A number of capping materials will therefore be reviewed from the point of view of biocompatability.

1.2.4.1 Zinc oxide eugenol

The most commonly used material for pulp capping in the past has been zinc oxide eugenol.
Glass and Zander (1949) reported no bridge formation under zinc oxide eugenol and a persistent chronic inflammation

at the site of the exposure. This is in agreement with Seelig, Fowler and Tanchester (1954), who described an inflammatory response with abcess formation when zinc oxide eugenol paste was placed in contact with pulps of monkey teeth. Sela, Hirschfeld and Ulmansky (1972), after capping rat pulps with zinc oxide eugenol, found inflammation and necrosis. This effect was even more prominent when the cement was used over pulps with an acute inflammatory reaction. This is in contradiction with the results of a study by Tronstad and Mjör (1972), who exposed and capped experimentally inflamed pulps with calcium hydroxide or zinc oxide eugenol. In this investigation the results of capping with zinc oxide eugenol showed some promise, if a pulp free of inflammation is an acceptable goal. This is in agreement with Weiss (1970), who stated that zinc oxide eugenol in direct contact with the pulp stimulated very little bridge-formation but was well tolerated by the vital pulp in an experiment of exposed sound monkey teeth.

Conclusion:
Consequently, the reaction of the pulp to zinc oxide eugenol appears to be uncertain.

1.2.4.2 Calcium hydroxide suspended in water

Controversial findings also exist about calcium hydroxide as a capping material.

Glass and Zander (1949) stated that when the healing of a pulp lesion is defined as the walling off of the exposure by new dentine formation, calcium hydroxide, within four weeks promoted, the healing of the pulp which then at that moment, was relatively free of inflammation.

Jeppesen (1971) correlated clinical findings with histo-

logical observations and found in 88 per cent of the pulp cappings that were considered succesful clinically no internal resorption and less than a moderate amount of lymphocytes and plasma cells in the histological preparations of the capped area. The formation of hard tissue over the exposure was described but not used as a criterion of success.

Pereira and Stanley (1981) capped exposed dog pulps with calcium hydroxide powder mixed with water and found no inflammation in 17 of the 24 pulps examined. The remaining 7 pulps showed chronic inflammation, ranging from mild to moderate. Calcified bridge formation was stimulated and maturated after 120 days. Nyborg (1955), also reported a 70 per cent success in capping human pulps with calcium hydroxide in water.

The findings of Tronstad and Mjör (1972) imply that calcium hydroxide has no beneficial effect of an inflamed pulp.

Paterson (1976), after experimenting with exposed rat molar pulps, found poor results, with over half the teeth having completely necrotic pulps.

According to Sawusch (1963), the percentages of successful pulpal cappings varied directly with the extent of the pre-operative carious lesion.

Thus, findings after application of calcium hydroxide suspensions in water on exposures are equivocal and this might be explained by taking the presence or absence of an initial inflammatory reaction into account and perhaps a variability in reaction of the various animal species used for experiments.

1.2.4.3 Calcium hydroxide mixed with other materials

Calcium hydroxide suspensions in water require a long time for film formation. For this reason products have been developed containing calcium hydroxide and materials which provide hardening within a reasonable period of time. The composition of these prescriptions differs from one manufacturer to another. One of the best known examples is Dycal.

The outcome of a successful capping with Dycal, according to Tronstad (1974), was similar to that with calcium hydroxide in water but there were striking differences in the reactions of the pulp, e.g. the typical necrosis seen after capping with calcium hydroxide was not induced by Dycal. On the contrary cells, capillaries and fibers were present in the area of reaction in the pulp capped with Dycal. The quality of the bridges induced by Dycal was apparently not inferior to the quality of bridges in pulps capped with calcium hydroxide.

Negm, Grant and Combe (1980) histologically evaluated two cements for pulp capping in caries-free human teeth. The powder of the first cement consisted of 25 per cent calcium hydroxide and 75 per cent zinc oxide which was mixed in equal parts with 42 per cent aqueous solution of polyacrylic acid. The powder of the second cement consisted of 50 per cent calcium hydroxide and 50 per cent zinc oxide which was mixed with the same fluid as in the first cement. Compared with Dycal, which was able to induce healing in 81 per cent of the cases, the percentage of successful healing of the pulps treated with the first and second cements was 88 and 91, respectively. Negm, Combe and Grant (1981), capping exposed pulps of rat molars, enlarged the experiment with a third cement,

the powder consisting of 75 per cent calcium hydroxide and 25 per cent zinc oxide, mixed with 42 per cent solution of polyacrylic acid. The pattern of healing and the tissue reaction under these materials was approximately the same, except that the zone of degeneration was generally less in thickness under the cement with the lower content of calcium hydroxide. All three materials were biologically acceptable and could be used as pulp capping materials.

Pitt Ford (1979) investigated another commercially available calcium hydroxide cement: Procal, which contains less calcium hydroxide than Dycal. Comparing the effects of Procal and Dycal in capping exposed dog pulps, he found similar response. However when monkeys were used in the experiments the results with Dycal were less favourable.

Pitt Ford (1980) also investigated the effects of MPC on the exposed pulps of monkeys and compared them with those of Dycal. After 3 months, bridge formation was not observed in teeth capped with MPC. Many of the pulps had a large zone of necrosis. The extent of inflammatory cells varied greatly and did not appear to be related directly to the amount of necrosis. The pH of MPC is much lower than that of Dycal. This could account for the differences in pulpal response. The results of capping with Dycal concurred with those of previous studies: a normal tissue and complete bridge formation.

The conclusion seems to be justified that the outcome of capping exposed pulps with cements containing calcium hydroxide is depending on the percentage of calcium hydroxide, which in its turn influences the pH of the cement. However, Watts and Paterson (1977), investigating various metallic compounds as capping agents on rat pulps, found no apparent relation between pulpal response and the pH

of these compounds.

1.2.4.4 Antibiotics

As studies in germ-free rats indicated that the presence of bacteria may be the most significant factor in prohibiting healing after pulp exposure (Kakehaski, Stanley and Fitzgerald, 1965), incorporation of antibiotics into pulp capping agents to eliminate pulp infection was investigated.

Baker and Mitchell (1969) examined whether the topical use of a broad spectrum antibiotic would aid the defense mechanism of the vital pulp in overcoming infection.They concluded that the pulps which were capped with starch containing antibiotic responded more favourably than the starch-capped controls. But a residual inflammation in the antibiotic-treated teeth remained, as observed after a experimental period of 90 days.

Gardner, Mitchell and McDonald (1971) using Vancomycin in combination with calcium hydroxide, two agents complementing each other, obtained a complete bridge with lack of inflammatory cells in the exposed pulps of monkey teeth after 30 days, which was more successful than with calcium hydroxide alone.

McWalter, El Kafraway and Mitchell (1976) used the antibiotic Keflin in combination with Dycal or Durelon but, besides killing bacteria, Keflin was so severely irritating that the pulp tissue was damaged irreversibly after 29 months.

Although antibiotics appeared to be anti-microbially effective and thus might contribute to the healing of the infected exposed pulp, the danger exists of a possible sensitization of the individual and of development of

resistant micro-organisms.(Barnes and Langeland, 1966; Page, Trump and Schaeffer, 1973; Reed Sayegh and Awwa, 1973). For these reasons the use of antibiotics must be reserved for diseases for which their use is an undoubted demand.

1.2.4.5 Corticosteroids

For the purpose of reducing inflammatory response of the pulp, corticosteroids were incorporated in pulp capping materials. Combined with calcium hydroxide, corticosteroids markedly reduced edema and inflammatory infiltrate in the rat. It also markedly reduced or eliminated necrosis of the tissue bordering the calcium hydroxide (Bhashar, Cutright and van Osdel, 1969).

Schmid, Gloor and Schroeder (1974) experimentally capped a group of exposed monkey teeth with a combination of Ledermix^{R} (containing a corticosteroid and an antibiotic) and calcium hydroxide. After 14 weeks the results seemed promising for this combination of antiphlogistic-, antibacterial and dentinogenetic agents.

Thus all authors ascribed an amelioration of the health of the pulp to the action of corticosteroids. However, Baume (1965), Langeland, Dowden, Tronstad, et al., (1971) and Patterson (1976) concluded that the positive effect of a corticosteroid may be the temporary relief of pain, but in the long run it does not prevent or remove inflammation. A chronic inflammation remains causing many pulps to die after long periods of time.

1.2.4.6 Polycarboxylate cement

McWalter, El-Kafrawy and Mitchell (1973) evaluated Durelon as a pulp capping agent in monkey teeth and found mild

inflammatory reactions, which were seldom accompanied by bridge-formation at the exposure site.

El-Kafrawy, Dickey, Mitchell et al., (1974) investigated a polycarboxylate cement - named P.C.A.^{R} - and also found only mild inflammatory reactions after 3 months, concomitant with little calcific bridging. They found P.C.A. ineffective in combatting bacteria.

Beagrie, Main, Smith et al., (1974) capped exposed monkey teeth with three different polycarboxylate formulations. They were all biologically well tolerated, showing very little inflammatory response 2 and 7 days after capping, but a wide range of responses after 32 days. Its innocuous effect might be due to a rapid rise in pH during setting - from pH 1.5 of the liquid polyacrylic acid to neutrality after setting - and its ability to complex with proteins that would limit its diffusion through the pulp tissue (Smith, 1971). Nevertheless its use for pulp capping was not recommended because the material was ineffective in combatting bacteria and in stimulating calcific bridging (El-Kafrawy, Dickey, Mitchell et al., 1974).

Addition of calcium hydroxide and antibacterial agents could enhance its value as pulp capping agent (Beagrie, Main, Smith et al., 1974). Whether a calcific bridge is needed, however, is an open question.

1.2.4.7 Cyanoacrylates

Capping with cyanoacrylates gave rise to some promising expectations. Bhaskar, Cutright, Boyers et al., (1969) capped exposed pulps of miniature swine with the material whereas Berkman, Cucolo, Levin et al., (1971) used it on human pulps.The tissue directly under the isubutyl cyanoacrylate retained its vitality and only a mild inflammation

was evoked. Compared with the effects of calcium hydroxide on the pulp, isobutyl cyanoacrylate used as pulp capping agent appears to be at least as effective. It has the added advantage of producing immediate stanching and its application is relatively easy (Bhaskar, Beasly, Ward et al., 1972). However, results from other experiments with dogs demonstrated it to be less favourable (Nixon and Hannah, 1972). Further study is needed.

1.2.4.8 Collagen Preparations

Albers, Neugebauer and Bull (1978) capped exposed dog pulps with Lyodura^{R} - a commercially available lyophilized. antigenically altered collageneous material - and fixed it in position with a cyanoacrylate wound dressing. After eight weeks the Lyodura capping resulted in hard-tissue bridge formation.

Dick and Carmichael (1980) covered exposed dog pulps with two types of collagen preparations, one highly porous, the other relatively non-porous. Their conclusion was that although the porous product was much better tolerated than the non-porous, calcium hydroxide is a more effective promotor of repair by hard-tissue bridge formation than the collagenous materials.

Albers (1980, 1981) histologically examined five exposed dog teeth capped with Lyodura, and out of a clinical investigation with Lyodura, three human teeth could be histologically examined. After 3 months there was hard-tissue bridge formation in the animal experiment but after 3 months in the human teeth the conductor function of the collagen had led to the growth of tissue between the partially formed bridge and the capping material. For that reason the application of Lyodura was not altogether successful.

1.3 Summary

Even with a clinical failure rate of approximately 25 per cent, the popularity of the Formocresol pulpotomy for exposed deciduous teeth is high. The reasons for this popularity can be the apparent clinical success of the procedure and the ease with which it can be applied. Both arguments, however, are debatable. The procedure of pulpotomy which has to be performed preferable under aseptic conditions, is relatively time consuming, requires skill and good coöperation of the young patient, conditions which seldom are met in general practice. The apparent clinical success of the pulpotomy has led to the claim of "vital" pulp treatment.The results of histological studies are not in agreement with such a claim. Studies (Rølling and Lambjerg-Hansen, 1978; Mejåre, 1979) have shown that the so-called "fixed" tissue is in reality a necrotic tissue that can act as an environment for bacterial growth and as a cause of chronic inflammation in the underlying vital part of the pulp and the periapical tissue, which can be a risk for the germ of the underlying permanent tooth.

A more biological approach could be capping of the pulp lesions with an agent which is able to promote healing of the pulp so that a sound pulp is attained. The reported investigations all started from the premise that the pulp tissue was not inflamed and even then only few materials appear to be biologically acceptable when in contact with the pulp. The effect of capping with Dycal polycarboxylate cements and cyanoacrylates gave rise to further development and investigation. Moreover, lack of agreement among research workers on the goal to be achieved - which largely revolves around the questions of the relevant criteria for the healing of an exposed pulp - interferes with the formu-

lation of a common point of view about adequate objectives for biological therapy.

Notwithstanding the seemingly few prospects of effectively promoting healing of a wounded pulp, as evidenced in the literature, an attempt will be made in the next chapter to devise a treatment which contributes to the healing of the pulp after exposure.

2. DESCRIPTION OF THE INVESTIGATION

2.1 Purpose of the investigation

Where Formocresol pulpotomy is applied clinically, three determinants come into play:

- The pulp is traumatized by the exposure;
- The pulpal wound surface is infected, either directly by bacteria of the carious lesion or indirectly by contamination during removal of the carious dentine;
- The pulp may be inflamed as a result of the toxins of bacteria in the carious lesion.

In present-day therapy, at least in pedodontics, the pulp is excised from the pulp chamber, Formocresol is applied to the root pulps, and the cavity is sealed. This therapy has a failure rate of 25 per cent (Chapter 1.1.3).

Surpassing the desirability of studying the background of the high failure rate of Formocresol pulpotomy and attempting to improve this therapy, a more logical aim is felt to be the recovery of healthy pulpal tissue in the pulp chamber.

To achieve this aim - obviously after removing the causative factor, the carious dentine, and relieving the patient of pain - the following desiderata should be realized:

a. To retain all pulpal tissue left after exposure.Thus, no pulpotomy is performed, but direct pulp capping.
b. To restore the pulp to a healthy condition. A healthy condition can be defined as a vital pulp, free of vascular inflammatory reaction (circulatory stasis, exudate and cells) and degenerative tissue changes. In other

words, the desired condition is one approximating, as closely as possible, the condition of the pulp prior to inflammation and exposure. It also means that no excessive amount of irritation dentine should form.

c. To restore the pulp to an unthreatened state. The meaning of this is threefold:
   1. The wound surface must be disinfected;
   2. The restorative materials being used must be acceptably biocompatible;
   3. The restorative materials should, ideally, prevent micro-leakage of bacteria past the filling material. If this prevention is complete, no hard tissue bridge is necessary at the site of exposure. With some filling materials, micro-leakage can occur and a hard tissue bridge might then be significant in excluding bacteria from the pulp.

Before direct pulp capping can replace clinical Formocresol pulpotomy, various animal experiments need to be made to gain an insight into how and to what extent the above-mentioned desiderata can be satisfied. The present animal experimental study does not go so far. Its more modest aim was to study:

- whether medication of the exposed inflamed pulp can promote recovery of a healthy condition and
- whether an unthreatened state of the pulp can be attained, at least in animal experiments.

2.2 Study design

During pulpotomy, as well as at the moment of exposure, the risk of bacterial contamination is high. Thus the pulp wound and the surrounding dentine have to be disinfected, in this sense that the amount of bacteria has to be dimi-

nished, so that the pulp tissue does not necessarily succumb to their attack.
Formocresol is a strong disinfectant containing 19 per cent formaldehyde and is used clinically in the Formocresol pulpotomy where disinfection is one of its effects. However, when disinfection can be achieved with agents containing lower concentrations of formaldehyde than Formocresol an answer had to be sought to the question how much the concentration of formaldehyde can be reduced, so that the negative effects of Formocresol no longer occur.
This question was studied in a pulpotomy experiment where the effects of lower concentrations of formaldehyde are compared with the tissue changes due to Formocresol itself. For this study pulpotomy was preferred to capping after exposure because noxious effects of the agents studied, if any, can be observed in the more or less cylindrical root pulp where coronally - the site of application - the most severe reaction can be expected, followed in apical direction by less severe tissue changes.

Whereas in the first part of this study, pulpotomy was performed, from this point onwards the pulps were exposed only (Desideratum a). If such agents are used as disinfectants immediately after exposure, their anti-microbial effectiveness should be studied. The reaction of the pulp to these agents was investigated (Desideratum c 1).

One of the requirements a sealant has to satisfy, especially when applied to an exposed pulp, is biocompatibility. This was studied for a number of commercially available dental cements (Desideratum c 2).

Microleakage of bacteria past a sealant would interfere with any healing of the traumatized pulp. A study was performed

to find a method that guaranteed freedom from micro-leakage over a medium-long period (Desideratum c 3).

To promote healing of the traumatized pulp in which an inflammatory reaction is present due to bacterial attack and/or exposure the application of an antiphlogistic might be significant. The concept of applying a controlled drug release system containing an antiphlogistic to the wound surface - at the bottom of the cavity - was worked out. The pattern of release of an antiphlogistic from a number of commercially available dental cements was studied in vitro.

In the above mentioned studies healthy pulps were used. This is in conformity with general practice when dental materials and medicaments are being tested biologically (Stanford, 1980). For an investigation of the effect of an antiphlogistic on the healing of an exposed pulp however, the use of inflamed pulps is more in agreement with the clinical situation, where a pulp has been exposed during the removal of carious dentine.
A study was therefore performed to investigate whether a 2-day infection of exposed dog teeth with Strep. faecalis provoked a moderate inflammatory reaction.

Finally, a study was made to obtain results after bacteria-tight capping of exposed infected pulps with:
- Cavit
- Cavit containing an antiphlogistic (Tantum)
- Durelon
- Durelon containing Tantum

The effect of Tantum in promoting healing of the pulp was studied (Desideratum b).

2.3 Results and discussion

In dentistry for children Formocresol pulpotomy is an accepted method of treating the exposed pulp of a carious tooth. Formocresol fixes the traumatized tissue and disinfects the wounded root pulps. These effects are achieved because Formocresol consists of 19 per cent formaldehyde (for comparison, histological fixation uses only a 4 per cent formaldehyde solution).

The reports in literature on the effects of Formocresol vary on the tissue changes observed histologically depending mainly on the period of action of the Formocresol. After application of Formocresol over an extended period, the histological changes include a total fixation of the pulp tissue. The damage is less Formocresol acts over 5 minutes only, in which case the apical part of the root pulp is vital, the middle part consists of necrotic tissue, and the part adjacent to the wound surface demonstrates histological fixation.

In an investigation on tissue fixation and response after the application of devitalizing pastes containing high percentages of formaldehyde, it was observed that those pastes provoked both stasis and inflammatory reaction, with the former enhancing the latter (Simon and van Mullem, 1978)

The presence of stasis in the circulatory system can be considered a life-threating factor for the pulp. From the point of view that healing of the wounded pulp is preferable to a questionable condition of a fixed tissue containing thrombi, the first object of the present investigation was to study the concentration of formaldehyde which does not give rise to stasis in the pulp and which also otherwise does not add any appreciable noxious effect to the damage resulting from the wound. For purpose of comparison,

Formocresol treated teeth were also studied.

The results of this investigation, described in Article I, show that a 5-minute application of Formocresol, Alcoformol 19/60 or 8.75/60 (prescriptions containing 19 per cent and 8.75 per cent formaldehyde resp.) produced an area of fixed pulp tissue adjacent to the wound surface and thrombi (in the two last mentioned prescriptions, cresol was omitted because an investigation by Ranly and Fulton (1976) revealed that cresol delayed the healing of the Formocresol treated pulp). Neither drug-fixed area nor thrombi were observed in pulps after a 5-minute application of agents containing 4 per cent or less formaldehyde.

Healing of the pulp tissue should not be jeopardized by thrombi, by areas of fixed tissue, and/or by appreciable amounts of necrotic tissue. If formaldehyde is to be maintained because of its disinfecting power, only a short-term application of agents with low formaldehyde concentration should be considered. This would maintain blood circulation - without stasis - and add little damage to that already inflicted by the pulpotomy.

As pulpotomy means loss of pulpal substance from the pulp chamber and, consequently, loss of nutritive, formative and sensory functions of that part of the pulpo-dentinal organ, the question arises whether this loss of substance could be avoided. If the coronal part is not removed - for the time being leaving aside the possibility of the presence of an inflammatory reaction in the pulp chamber - a disinfectant will certainly be required. Will an agent, containing one of the lower concentrations of formaldehyde mentioned above then be effective enough as disinfectant ?

Article II describes a study of the effectiveness of two disinfectants: AF 1/10 and AF 3/20, containing 1 per cent and 3 per cent formaldehyde resp. and their action on exposed, contaminated pulps of monkey teeth.

This study consists of two parts: an in vitro and an in vivo experiment.

In both experiments the cavities were infected with a freshly prepared aqueous 10^6/ml suspension of Strep. faecalis for 5 minutes. Strep. faecalis was chosen because this bacterial species appeared to be rather resistant to disinfection (Wesley, Marshall and Rosen, 1970) and is always present in the flora of the mouth. The way of infection mentioned served as a model for bacterial contamination, as can happen clinically when a pulp is exposed during the removal of carious dentine.

After the 5-minute infection, the cavities were immediately disinfected with AF 1/10 or 3/20.

The cavities were of standard size, shape and direction. Cylindrical cavities (Ø 1 mm) were drilled perpendicular to the pulp. These characteristics were chosen to keep the trauma of drilling through the dentine as small as possible and to obtain standardized exposures. A disadvantage of this shape is that they are less accessible for the introduction of liquids than the deep cavities in clinical practice where exposures of the pulp happen to occur. Ethyl alcohol was a component of the disinfectants to lower their surface tension and to improve their wetting property. For ethical reasons an in vitro experiment preceeded the in vivo study. It closely imitated the clinical situation and was performed to obtain an indication of what could be expected on the effectiveness of the disinfectants in vivo under usage conditions.

In the in vitro study extracted sterilized human teeth, in which the pulp tissue has been replaced by an agar medium, were used. Cavities were drilled, and infection, immediately followed by disinfection, was performed in the way described above.

Bacteria were cultured 3 days after disinfection and sealing with Cavit. The results demonstrated both disinfectants to be equally effective.

In the in vivo experiment the presence of bacteria was scored using Brown and Brenn stained sections of the histologically processed teeth. Regarding the presence of bacteria, the results of this experiment after 14 days supported the results of the in vitro investigation.
Beside Brown and Brenn stained sections, other sections were stained with haematoxylin-eosin for the evaluation of the pulp tissue reaction.

The results of the evaluation of the tissue reaction revealed that both disinfectants were well tolerated by the exposed pulp. An explanation of the high percentage of teeth that scored positive for bacteria after disinfection could be the presence of an air bubble in the narrow, deep cavity, which would prevent the action of the disinfectant. In the in vivo experiment, microleakage of the sealant could be another explanation of this phenomenon.

The conclusion from this study, which is presented in Article II, is that AF 1/10 and AF 3/20 are equally effective and well tolerated by the pulp.

At the time the carious dentine is removed, the pulp tissue can be inflamed as a consequence of toxins of bacteria which are present in the carious dentine. It is questionable whether the inflamed pulp tissue is able to heal without any help. An antiphlogistic released from a carrier could promote healing after the exposure is capped with

that material. However, cytotoxicity of the carrier itself can negatively influence this process. So, one of the demands to make upon a carrier is biocompatibility. Other demands are enough mechanical strength to withstand forces related to the application of the permanent filling material, and properties such as hydrophilicity which is a determinant factor on the release of the drug.

Commercially-available filling materials could fulfil these requirements, and for economical reasons, they were also the first choice as carriers.

To obtain information about what material, out of a group of five, could be chosen for an in vivo investigation, three hydrophilic filling materials (ZnOE, Cavit-W and Durelon) and two hydrophobic (Concise paste and Nimeticap) were tested for their biocompatibility by means of an agar overlay technique and human skin fibroblasts. In this in vitro experiment a standard mix of zinc oxide eugenol served as reference material.

Of these cements, Nimeticap appeared to be significantly less cytotoxic, when compared with Cavit, Durelon and Concise paste. Among the three last-mentioned materials, no significant differences were revealed. ZnOE appeared to be the most toxic of the cements investigated. For the in vivo test on biocompatibility Nimeticap was chosen on the basis of the in vitro results. Moreover, of the group of three cements which did not differ in vitro, Cavit was chosen, because of its use in previous investigations.

Because of a shortage of monkeys, part of this study had to be conducted on young permanent dog teeth. Although differences have been reported in pulp tissue reactions in dogs and monkeys, to various noxious influences, statistical testing in this study revealed no significance. Therefore, the results in dog and monkey teeth, if capped with

the same sealant, were pooled. Obviously, pulp tissue reactions to Nimeticap and Cavit were studied only in teeth that were negative for bacteria.

The results given in Article III indicated that, from the point of view of biocompatibility, Cavit appeared to be the most favourable filling material.

The results of this study contributed to the choice of materials to be tested for drug release in vitro.

However, prior to a general discussion on controlled drug release and an experiment concerning drug delivery, another problem will be dealt with. This problem relates to bacteria-tight sealing of experimental cavities and, as such, has wider relevance than for sealing cavities after exposure only.

For the study of pulp tissue reactions to materials and medicaments, teeth which were positive for bacteria had to be excluded in order to exclude reactions of bacterial origin. In the previous investigations on the biocompatibility of capping material, high percentages (up to 50 per cent) of the teeth appeared to be Brown and Brenn positive. Two factors can be supposed to cause this trouble:

1. contamination of the pulp tissue by bacteria during exposure,
2. microleakage of bacteria during the experimental period.

Contaminating bacteria can be reduced by disinfection (Article II). The problem of microleakage should be solved, not only to save a number of experimental animals, but also to save histotechnical work.

From clinical experience it is known that the aim of microleakage-free sealing is not an easy one. Much research with a clinical aim has been done on preventing the effect of microleakage past filling materials. Some of these in-

vestigations are:

- The role of the smear layer on the prevention of transport of fluids through the cut dentinal tubules (Dippel, 1980).
- The effect of introducing varnishes in the cavity before restoration of the teeth with amalgam (Dolven, 1966; Edwards, 1978).
- Antimicrobial action of liners per-sé (Fisher, 1972).
- The adhesive attachment of polymer systems to etched enamel (Arends, 1979).
- Although attachment to dentine is still a problem according to Arends (1979), Fusayama (1980) claimed that Clearfil demonstrated leakage-tight polymer adhesive attachment to dentine after etching.

In addition, Fusayama claimed that the material was non-irritating to the pulp. If histological examination of short- and long-term experiments performed by independent investigators can support this claim of biocompatibility, this method would solve the problem of micro-leakage for those cases where a composite filling material can be applied clinically.

The above-mentioned polymer material was not yet available at the start of the present study and therefore could not be scrutinized for microleakage and biocompatibility. Thus, another way of solving the problem of microleakage in experimental cavities over middle long-term periods was taken.This was already done in an in vitro investigation by The, van Mullem and Plasschaert (1982).

To investigate the sealing properties of bondings, they performed an in vitro experiment on caries-free human teeth in which 2 mm deep cavities were drilled. The cavities were filled with Cavit-W or gutta-percha point sections. In a number of other teeth no material was intro-

duced. All cavities were covered with a layer which consisted either of a chemically polymerizing bonding (Concise bond) or of a UV polymerizing bonding (Nuva-Seal or Estilux glaze). During the test period of 5-8 weeks, the temperature of 37°C was lowered to 8°C twice daily for 15 minutes to simulate temperature changes to which teeth in experimental animals are exposed during feedings. As tracer for microleakage, crystal-violet, being a small molecule, was chosen. If such a small molecule did not penetrate, penetration of bacteria could not be expected either.

The result of this study was that sealing with the UV polymerizing Estilux glaze, after etching of the surrounding enamel was effective. Micro-leakage was prevented over a period of 8 weeks, whether the cavities were filled with gutta-percha or were empty.

On the basis of the fact that in animal experiments, edge strength, impact strength and other factors can influence this result, an in vivo experiment was carried out. Exposed dog pulps were sealed with Cavit and a layer of a chemically polymerizing bonding (Concise, experimental period 14 days) or a UV polymerizing bonding (Uvio-Bond, experimental period 14 and 42 days) was applied over the sealant after etching of the enamel.

Statistical testing revealed that - after 14 days - the UV polymerizing Uvio-Bond achieved a better bacteria-tight seal of the deep cavities than the chemically polymerizing bonding. After the middle long term of 6 weeks, no teeth demonstrated microleakage, as studied by Brown and Brenn stained tissue sections. (Article IV).

The following sections deal with the concept which, it was hoped, would solve the problem of providing appropriate help towards the recovery of the traumatized pulp. First some remarks on drug release from biomaterials will be made. These will be followed by a discussion of the results obtained in studying drug release from some dental cements (Article V).

Regarding the delivery of the drug at the desired site - the wounded tooth pulp - several considerations have to be taken into account. The conventional way of drug administration, by way of the mouth or the vascular system, mostly involves repeated doses of such amounts of the drug as is necessary to obtain an effective level at the site of interest. It also brings along peaks and valleys in the drug level of the blood because, after an administration, first the drug has to become available and/or to be distributed over the body, resulting in an increase of its concentration in the blood, followed by a decrease (e.g. by chemical reaction or excretion). Moreover, an effective dose of an antiphlogistic at the intended site might also be effective at sites of physiological inflammatory reactions where its action is not desired. For these reasons injection or oral administration of an antiphlogistic to decrease an inflammation in a pulp as an initial step towards healing did not seem to be a first-choice method.

The unintentional exposure of the pulp now becomes an advantage. The deep cavity offers the possibility of applying a suitable biomaterial containing an antiphlogistic at a location where the drug is desired: the inflamed pulp and/or the pulp wound. Continuing drug release from such a carrier - preferably in a way in which the amount released is directly proportional to time - avoids peak-and-valley levels in the pulp. Moreover, if an effective level is

achieved in the pulp, the drug, after being carried away by the blood, will very probably be - by dilution - at an ineffective level elsewhere in the body.

The idea of incorporating a medicament in a polymeric biomaterial to obtain release over a prolonged period is not new in medicine. In 1965 Desai, Simonelle and Higucki had already dispersed a solid drug in polyethylene and had studied release.

To our knowledge, however, the application of the concept of controlled release of an antiphlogistic from a carrier is new in the situation where an exposure of the pulp is capped.

In this case, preferential to sustained release, controlled drug release should be aimed at. Sustained drug release can be defined as a delivery over a number of hours only, as takes place with tablets and ointments. This is contrary to controlled release where delivery lasts one day or longer. It was felt that a beneficial effect of a suitable antiphlogistic would require action over a period of at least some days, but preferably over a period of one week or more.

With regard to the application of a controlled drug release system to an exposed pulp, some potential advantages can be mentioned:

a. Harmful side effects from systemic administrations can be avoided by topical administration from a controlled drug release system.
b. The drug level in the pulp can be maintained within a therapeutically desired range over a certain period of time.
c. Antiphlogistics that have short half-lives when given systemically may not be degraded in the pulp when delivered directly to it.

d. Drug administration by such systems may be less expensive than when given in larger doses systemically.

A number of possible disadvantages should also be considered:

a. Components of the carrier material which leak out of the system may be bio-incompatible to the pulp tissue.
b. If the carrier is biodegradable, the degradation products might be toxic.
c. Certain prescriptions can be expensive to produce industrially.

With these disadvantages in mind, the present investigation selected as carriers those dental cements which were known to be acceptably biocompatible. These cements are not biodegradable and the simple addition of an antiphlogistic to them will not tremendously increase their costs.

Some examples of controlled release systems, the release duration of which ranges from 3 days to 1 year, are the following (Langer and Peppas, 1981):

Commercially available are:

a. Ethylene-vinyl acetate containing pilocarpine. It is implanted in the conjunctiva for treatment of glaucoma and releases over 1 week.
b. Ethylene-vinyl acetate containing progesterone is used for birth control. It is implanted in the uterus and releases over a period of 1 year.
c. Scopolamine incorporated in a microporous membrane can be implanted in the skin. It is used against motion sickness and releases over 3 days.

Studied in clinical trials are:

d. Steroids incorporated in various polymers which are implanted subcutaneously for birth control and act over period of 6 months to 1 year.

e. Testosterone in silicone against prostate cancer is implanted subcutaneously and releases over a period of 1 year.
f. Hydroxyethyl methacrylate-methyl, methacrylate copolymer to which sodium fluoride is added. Serving to prevent caries it is attached to the surface of molars and releases its drug over a period of 6 months (Mirth, 1980).

The controlled release polymeric systems which are either in clinical use or have been investigated in clinical trials or in in vivo and in vitro experiments can be classified in four groups:

a. Chemically-controlled systems.
Belonging to the chemically-controlled systems are the biodegradable systems and the pendant chain systems.

In the biodegradable systems, the drug is distributed uniformly in the carrier. The drug is not released by diffusion, but is delivered to the extent with which the carrier is degraded by hydrolytic or enzymatic cleavage. The end result is that nothing is left at the site of application.
For this reason these systems were not the first choice in the present study. An empty space would be left - after degradation - between the dentine cavity floor containing the exposure and the permanent filling material with which the carrier, used as capping material, must be covered. Such an empty space can add to loosening of the permanent filling material.

In the pendant chain systems, the drug is chemically attached - sometimes via a spacer group - to the long chain polymer molecules which serve as backbone for the drug molecules.
From this group of polymers only those which are in-

soluble and non-biodegradable could be considered for application after exposure.

Release is dependent on hydrophilicity of the polymer and takes place by hydrolysis or enzymatic cleavage of the bond between drug and polymer backbone. The consideration that the chemical synthesis of drug systems belonging to this group would require a great deal of effort led to the decision to leave them aside for the time being.

b. Swelling-controlled systems.

In the swelling-controlled systems the drug is distributed uniformly in the polymer matrix. No diffusion is possible initially, but when the polymer absorbs water, the matrix swells, becomes rubbery and diffusion starts. For the application envisaged, neither swelling nor a rubbery consistency was wanted. A rubbery mass underneath the permanent filling material might lead to loosening of the filling. Swelling of the material would most probably produce an effect in the direction of the pulp and cause another trauma.

c. Magnetically-controlled systems.

Small magnetic beads and the drug are uniformly distributed in the polymer carrier. When the system is in contact with water,some drug release occurs and is controlled by diffusion. If an oscillating magnetic field is applied, drug release is greatly increased. Obviously, application of a magnetic field to a patient's tooth is out of question.

d. Diffusion-controlled systems.

Belonging to the diffusion-controlled release systems are the reservoir and the matrix systems.

The reservoir systems (membrane devices) consists of a core containing the drug and a surrounding polymer

layer containing no drug. This outer layer is rate-limiting. Most of the earlier mentioned release systems belong to the reservoir type.
The polymers used for these systems are relatively biocompatible, are generally not biodegraded, are suitable for the permeation of low molecular weight (< 600) substances, and can be used to design systems demonstrating release where the amount is directly proportional to time (zero-order release kinetics). This is achieved by loading the core with powdered drug. During the time powdered drug is present, the drug concentration in the core is the saturation concentration and zero-order release occurs (Langer and Peppas, 1981).

All characteristics mentioned above can be considered advantages for an application at the wound after exposure. However, the dimensions of the existing devices (e.g. spheres, capsules, hollow tubes) seem to obviate the application under consideration here.

In matrix systems, the drug is uniformly distributed in the solid polymer and drug diffusion is controlled by the polymer matrix (carrier). Such systems can easily be prepared in the laboratory by mixing the drug with a paste-like filling material or by adding the drug to a component of a composite filling material. The release from these systems, however, is generally not of zero-order. An example of this can be found in the work of Fu, Mayer and Hagemeier (1978). Release of hydrocortisone from ethylene-vinyl acetate copolymer matrices was demonstrated to be proportional to the square root of time, instead of being proportional to time (zero-order release).

The requirements of the carrier to be applied to the exposure in a deep cavity include:

- It should be mouldable to facilitate a good fit to the floor of the cavity.
- It should be easy to handle.
- It should have sufficient strength after polymerization e.g. to withstand condensation of amalgam.
- It should be non-biodegradable.
- It should have favourable biocompatibility.
- It should be self-sterile or easily sterilizable.
- It should allow as near zero-order drug release as possible.

Most of these requirements combined with the sterile - at least to start with - to work with a system which can be easily prepared, led to the choice of diffusion-controlled systems for drug release, and more specifically to matrix systems.

To further facilitate preparation of the medicament, commercially-available dental cements were chosen. Some hydrophilic (Cavit and Durelon) and some hydrophobic (Ketac, Nimeticap, Visiodispers[+)] *dental cements were included in the study represented in Article V.*

As antiphlogistic drug, the non-corticosteroid Tantum[x)] *(benzydamine hydrochloride) was chosen).*
Besides its general antiphlogistic action, it combats post-operative traumatic swelling and exerts some anaesthetic action.

After addition of the powdered substance to the cements, release was measured in buffer solutions UV photometrically.

+) Courtesy ESPE, Seefeld/Oberbay., GFR.

x) Courtesy N.V. Organon, Oss, The Netherlands.

The results described in Article V suggest that for studying biological effects of Tantum release, Cavit-W should be used as matrix if short-term release (2 days) is envisaged and Durelon of the effects of long-term release (7 days) are to be studied.
Although no zero-order release was observed, it was still felt justified to perform an animal experiment under usage conditions to study the effect of Tantum on the pulp.

Up to this point healthy teeth only had been used in this investigation. In the clinical situation, an exposed pulp is more often than not an inflamed pulp. So the aim of capping is to heal an inflamed exposed pulp. It was therefore more realistic to study the influence of disinfection and the application of medicament to teeth with inflamed pulps. In healthy animal teeth, inflammation first had to be induced to obtain pulpitis.

Literature reports few investigations about inducing pulpitis. Mjör and Tronstad (1972), using healthy monkey teeth, drilled cavities, the floor of which was in the innermost third of the dentine.
Inflammatory reaction was provoked by:

1. placing soft carious human dentine on the bottom of the cavity, followed by sealing with amalgam,
2. sealing with gutta-percha, or
3. open contact with the oral environment.

Of these three methods, only the first two histologically appeared to give reactions which were compatible with the aim of the investigation: inducing a standardized and reproducible pulpitis. Lervik and Mjör (1977) extended this investigation and ascertained the period in which severe inflammation of the pulp tissue was induced. Where human carious dentine was placed on the bottom of the cavities, this was 2-5 days. With gutta-percha, 2-5 days

gave rise to a slight reaction of the pulp, 8 days to a moderate reaction and 10 days to a severe reaction.

Infection of exposed pulps by introducing a suspension of Strep. faecalis for 5 minutes has been reported by Isermann and Kaminsky (1979). After 3 days the cavities were re-entered and appeared to be positive after culturing, but the effect was not evaluated histologically. *Such an evaluation was the purpose of the investigation described in Article VI.*

Introduction of Strep. faecalis in cavities of dog teeth with exposed pulps and sealing with gutta-percha resulted - after 2 days - in pulps which on the average demonstrated histologically a pulpitis of moderate degree. There were no teeth which scored no reaction.

The method presented here offers three important characteristics for experimental work:

1. A group of teeth demonstrating an average pulpitis of a moderate degree and with no pulps scoring no reaction is a favourable starting-point for the study of treatments aimed at healing.
2. The brief period of only two days for the induction of pulpitis offers a convenient start for an experiment.
3. The presence of bacteria at and near the wound surface is in close agreement with the clinical situation.

In the foregoing, the discussion concerned an experimental model which was developed to study the effect of medicaments in vivo under usage conditions. This model includes:

- *A disinfectant. An agent with favourable biocompatibility (Article I and II) and sufficient anti-microbial power (Article II) was found to be at least suitable for animal experimentation.*
- *Drug carriers. Two dental cements were found which demon-*

strated an interesting pattern of release of an antiphlogistic (Article V). Both showed a favourable biocompatibility (Article III).

- *A sealant. A bonding was found which enabled a bacteria-tight sealing of the cavity for the middle long term (Article IV).*
- *Pulpitis. A way of intentionally inducing bacterial pulpitis was found (Article VI).*
  Rather than attempting to optimalize this model, it was decided to direct activities to obtaining evidence for the question whether - after exposure - controlled release of an antiphlogistic from a carrier - applied to the cavity floor - would favourably influence an inflamed pulp.

The purpose of the investigation described in Article VII was to study the influence of the antiphlogistic drug Tantum on the tissue reaction in an exposed and inflamed pulp.

The pulp was infected to create an inflammatory reaction of moderate degree and to simulate clinical conditions as closely as possible. Obviously, disinfection was performed before the drug was applied.

In the belief that a pulse administration of a non-toxic amount of the drug would be ineffective and to avoid repeated administration, preference was given to a controlled release system delivering the drug at the site where its action is desired. Carriers used for the Tantum were Cavit and Durelon. The leakage pattern of each Tantum-containing carrier was studied before in vitro (Article IV). Experimental periods were 2 and 7 days.

Cavities were sealed with either one or the other of the two carriers, both of which contained Tantum or not.

There were thus four groups of teeth, the tissue sections of which were evaluated for bacteria or inflammatory reaction and necrosis after appropriate staining.

Of the 90 dog teeth used for this investigation, 11 per cent proved to be positive for bacteria after 2 and 7 days. This could not be explained by microleakage past the Cavit or Durelon, because a layer of Uvio-Bond has been applied over the fillings and the surrounding enamel. This method revealed a prevention of microleakage for at least 6 weeks (Article IV). The brief period of time (5 minutes) during which the disinfectant was applied might be the cause of this result. The disinfecting power of a 5-minute application of the agent had been tested for its effectiveness in combatting contaminating bacteria after exposure. In the present experiment the bacteria might have become located at sites not easily reached by the disinfectant.

To eliminate reactions of bacterial origin in the results of the four experimental groups, only histological sections of teeth that were negative for bacteria were used. These sections were scored for inflammation and necrosis at the wound surface and at a site 2000 µm apically.

Statistical comparison between the scores for the wound surface and those of the site 2000 µm apically revealed that 8 out of 16 p-values indicated (near) significance. The direction of (near) significance was equal: a less severe tissue reaction at 2000 µm than at the wound surface The meaning of these significances is that the pulpal tissue over a length of 2000 µm is involved in the tissue reaction to a lesser degree in these significant cases.

Another result of this investigation was that although it was assumed that Durelon would provoke a more severe tissue reaction than Cavit, statistical testing of results obtained from the capping with Cavit or Durelon, both

without Tantum, did not support this assumption.

It was concluded that, for the time being, there is no reason to believe that Durelon is more toxic to the exposed pulp than Cavit.

The inflammatory reaction to the operative trauma and the capping with Cavit appeared to decrease over the first 7 days after exposure even without addition of the antiphlogistic Tantum.

Where Tantum was added to Cavit, the inflammatory reaction at the wound was significantly less severe after 2 days than when capped with Cavit without Tantum. After this initial suppression of the inflammation, no further influence of the addition of Tantum could be ascertained. Thus, the end result, after 7 days in this study, appeared to be similar for Cavit and Cavit that contained Tantum.

The results obtained when the release of Tantum from Cavit was studied in vitro (Article IV) appeared to have predictive value.

The necrosis scored as reaction to capping with Cavit only was more severe after 7 days than after 2 days, both at the wound surface and at a site 2000 µm in apical direction. If studies with longer experimental periods were to reveal further increase in necrosis, this would be an unfavourable effect of capping with Cavit without drug.

Using Cavit containing Tantum for capping, statistical testing revealed a slight indication that the addition of Tantum reduced the necrosis during the period between 2 and 7 days.

In contrast to the results obtained after capping with Cavit, no significant reduction of the inflammatory reaction of the pulp tissue could be ascertained after capping with Durelon only, after 2 and 7 days, and neither was there an increase or decrease in necrosis.

The addition of Tantum to Durelon resulted in a nearly significant reduction of the inflammatory reaction over the period from 2-7 days and also, after an initial enhancement of the necrosis, in a significant reduction of necrosis at the wound surface and a nearly significant reduction 2000 µm apically.

A study of the controlled release of Tantum from Durelon in vitro (Article IV) showed that during the first two days Durelon released Tantum distinctly less than Cavit, but the release continued, although more slowly, over the period from 2-7 days. This phenomenon could account for the reduction of the inflammatory reaction and the necrosis over the period from 2-7 days.

Nevertheless the end conclusion of this study is that there is evidence of the effectiveness of Tantum - delivered from a controlled release system - in reducing inflammatory reaction and necrosis in exposed inflamed dog pulps. Release studies have to be continued in both short term and longer term (>7 days) experiments, using various concentrations of antiphlogistics and various degrees of inflammation to indicate more definitely whether complete healing can be achieved.

2.4 Remarks regarding future investigations

In endodontics, the tendency is to reply on complete endodontic treatment, and in pedodontics, on pulpotomy. But with the removal of the pulp, its nutritive, formative, and proprioceptive functions are lost. Pulp capping is performed only when nothing is available.

It is felt that if an opportunity arises for a biologically justified (direct) pulp capping, the natural healing power of the inflicted pulp must be given a chance.

The findings in this thesis - on disinfection, controlled drug release from a biomaterial, and bacteria-tight sealing - offer perspectives.

Items for future consideration are:

- Replacement of the disinfectant containing formaldehyde, which was used in the present study (AF 1/10) by a non-formaldehyde disinfectant). In recent years it has become the trend to replace all agents containing formaldehyde because of the substance's immunogenic potential. It might be wise to follow this trend.
- Incorporation of the disinfectant in the controlled release system to be used for the antiphlogistic. A longer duration of its actions could be obtained.
- Study of various controlled release systems to obtain zero-order release of several antiphlogistics in various concentrations over a period of 7 days or more.
- Experiments on actual drug level in the pulp to relate to pharmacological data.
- Experiments under usage conditions must be followed by clinical trials.

If favourable results could be obtained, the scope of application of the medicated capping material envisaged might be wider. Such capping materials might favourably add to the healing potential yet present in the pulp, not only after exposure, but also in deep carious lesions, whether in deciduous or permanent teeth.

This thesis deals with a study of the promotion of healing of exposed inflamed pulps, in which factors such as disinfection - as compatible as possible with the pulp tissue - and controlled release of an antiphlogistic from a well tolerated carrier, play a role.

Chapter I summarizes the many papers, both clinical and experimental, on the two main methods of treating exposed pulps: pulpotomy and pulp capping. Both methods claim to preserve the vitality of the (remaining) pulp.

To achieve this end, chemical agents are applied. The results, which sometimes closely correlate with the chemical nature of the agents used, are discussed. Most of the results appear not to fulfil the expectation, i.e. to maintain the vitality of the pulp tissue. Materials, initially demonstrating promising effects, gave rise to further investigation, but for the moment no biologically acceptable method of treatment has been developed on the basis of one or more of these agents.

Conclusion: As a consequence of the lack of acceptable reasons for choosing a biological therapy for treatment, the general practitioner prefers non-vital methods, disregarding their biological consequences. On exposed pulps of deciduous teeth Formocresol pulpotomy is performed. The treatment of exposed permanent teeth with incomplete apexification is temporary capping with calcium hydroxide after pulpotomy, followed by pulpectomy when apexification is completed.If the pulp of a fully developed tooth is exposed, pulpectomy is carried out immediately.

Chapter II formulates the purpose of the study, describes the study design, and presents the results and the discussion.

It was conceived that - after exposure - three desiderata have to be fulfilled:

- to retain all pulp tissue,
- to restore the pulp to a healthy condition,
- to restore the pulp to an unthreatened state.

The present study does not go so far that a clinically applicable treatment is attained; its more modest aim was:

to study whether medication of the exposed inflamed pulp can promote recovery of a healthy condition and whether an unthreatened state of the pulp can be attained, at least for animal experimentation, with the use of a pulp capping procedure.

This aim was subdivided into the following studies:

- on disinfection,
- on biocompatible capping materials,
- on bacteria-tight sealing of the cavity,
- on drug release, because it was felt that the pulp's natural power to heal has to be aided by an antiphlogistic,
- on induction of an inflammatory reaction in healthy experimental pulps,
- on the influence of an antiphlogistic on exposed inflamed pulps.

Descriptions of experiments for these subdivisions are added to this thesis: Articles I - VII.

Article I compares the histological results obtained after Formocresol pulpotomy with those after pulpotomy using agents containing lower concentrations of formaldehyde.

Conclusion: Short-term application of agents with low formaldehyde concentrations add little trauma to that of the pulpotomy and cause no circulatory stasis by thrombus-formation.

Article II describes the investigation into the power of agents with low-formaldehyde concentrations to disinfect bacterially exposed contaminated pulps. Also studied was their tissue compatibility.

Conclusion: The two disinfectants investigated were effective and well tolerated by the exposed pulp.

Article III describes the in vitro and in vivo studies of the biocompatibility of some dental cements as capping agents.

Conclusion: Cavit appears to be a more favourable capping material than Nimeticap, whereas Durelon appears in vitro to be of similar biocompatibility as Cavit.

Article IV describes an investigation into bacteria-tight sealing of exposed pulps. The necessity for this experiment arose from the study on the capping of exposed pulps (Article III) of which too large a number was positive for bacteria. Microleakage of the sealing material is known to be one of the factors that cause severe inflammation of the pulp tissue.

Conclusion: Sealing the cavity with Cavit and covering the Cavit and the surrounding enamel with UV polymerizing Uvio-Bond appears to be bacteria-tight in animal experiments of a middle long term (42 days).

Article V describes the pattern of drug release from some dental cements. This in vitro experiment was performed to obtain information about the expected differences in release from hydrophilic and hydrophobic cements. The results

indicated which materials could be expected to release the antiphlogistic Tantum in a way that could help to reduce the inflammatory reaction of the exposed pulp.

Conclusion: The difference in release pattern of Cavit and Durelon makes them suitable for in vivo investigation into the influence of Tantum on the inflammatory reaction in exposed inflamed pulps.

To study in vivo the effect of an antiphlogistic released from dental cements as carriers of the drug, an experimentally induced, standardized pulpitis was required. A method of inducing this pulpitis is described in Article VI.

Conclusion: The introduction of an aqueous 10^6/ml suspension of Strep. faecalis in the cavity of the exposed pulp results in an approximately moderate inflammatory reaction after two days.

This method of inducing pulpitis was used in the investigation described in Article VII.

Article VII describes an experiment in which the teeth are disinfected - two days after infection with Strep. faecalis - and capped with cements containing Tantum.

The influence of the controlled released antiphlogistic on inflamed dog pulps was evaluated histologically.

End-conclusions: With a capping procedure, medication of the exposed inflamed pulp by means of a controlled release of Tantum from a carrier is effective in promoting the recovery of a healthy condition.

In addition, an unthreatened state of the pulp can be attained, at least for animal experimentation, by sealing the cavity with the biocompatible Cavit and covering the Cavit and the surrounding enamel with Uvio-Bond.

In dit onderzoek wordt nagegaan of de genezing van een geëxponeerde en geïnfecteerde pulpa bevorderd kan worden door een zo bio-compatibel mogelijk desinfectans te gebruiken en door een antiphlogisticum toe te dienen door middel van een systeem van gecontroleerde afgifte uit een carrier.

In hoofdstuk I wordt een globaal overzicht gegeven van de literatuur over zowel klinische als experimentele onderzoeken, met betrekking tot twee methoden om een geëxponeerde pulpa te behandelen: de pulpotomie en de directe overkapping. Elk van beide methoden heeft zijn eigen aanhangers en van elk van beide wordt beweerd dat daarbij de vitaliteit van de pulpa - in het geval van pulpotomie van het resterende deel van de pulpa - behouden blijft.

De resultaten hangen soms nauw samen met de chemische samenstelling van de cementen waarmee de wond wordt afgedekt. De meeste van deze cementen kunnen niet voldoen aan de primaire eis: het in stand houden van de vitaliteit van de pulpa.

Hoewel er veelbelovende resultaten met sommige materialen zijn bereikt is een biologisch verantwoorde methode op dit moment nog niet voorhanden: d.i. een methode waarbij alle factoren die het van nature aanwezige vermogen tot genezen van de pulpa kunnen belemmeren, gereduceerd en liefst geëlimineerd worden.

Conclusie: Niet gemotiveerd door éénsluidende onderzoekresultaten verkiest de algemeen practicus een niet-vitale methode boven een biologisch verantwoorde therapie.

Voor het melkgebit valt de keuze dan op de Formocresol pulpotomie en voor het blijvende gebit op de totale extirpatie, de pulpectomie, zij

het dat als tijdelijke oplossing, in het geval dat de wortels nog niet afgevormd zijn, soms een pulpotomie met calcium hydroxyde wordt toegepast. Als de wortels zijn afgevormd wordt deze methode alsnog gevolgd door een pulpectomie.

In hoofdstuk II wordt het doel van het onderzoek beschreven, de verschillende onderdelen waaruit het onderzoek bestaat en wordt ingegaan op de gekozen volgorde. Tevens worden onder weglating van "Materiaal en Methoden", om het overzicht zoveel mogelijk te behouden, resultaten en discussie van de onderdelen van het onderzoek in het kort weegegeven.

Bij het onderzoek werd ervan uitgegaan dat - na exponatie - aan drie desiderata voldaan zou moeten worden:
- er moet niet méér pulpaweefsel worden opgeofferd,
- de pulpa moet opnieuw in een gezonde conditie worden gebracht,
- alle factoren die alsnog deze conditie zouden kunnen bedreigen moeten worden geëlimineerd.

Het huidige onderzoek gaat niet zover dat een klinisch toepasbare methode is gevonden. Een bescheidener doel werd nagestreefd:
In dierexperimenten nagaan of met behulp van medicatie via pulpa overkapping het herstel van een gezonde toestand van de geëxponeerde en geïnfecteerde pulpa bevorderd kan worden en nagaan of de factoren die de pulpa na de exponatie bedreigen geëlimineerd kunnen worden.
Om dit doel te bereiken werden deelonderzoeken gedaan op het gebied van:
- Desinfectie
- Biocompatibele afdekkingsmaterialen
- bacterie-dichte afsluiting van de caviteit

- Afgifte van een medicament uit een carrier. De noodzaak lijkt aanwezig om het van nature aanwezige vermogen tot genezen te ondersteunen met behulp van een antiphlogisticum.
- Opwekken van een ontstekingsreactie in gezonde experimentele pulpae.
- De invloed van een antiphlogisticum op een gezonde geëxponeerde, geïnfecteerde pulpa.

Deze experimenten worden beschreven in de artikelen I t/m VII.

In artikel I worden de histologische resultaten na Formocresol pulpotomie vergeleken met de resultaten na pulpotomie, waarbij desinfectantia met lagere concentraties formaldehyde worden gebruikt.

Conclusie: Het appliceren gedurende een korte tijdsperiode van agentia met een lage formaldehyde concentratie vergroot het trauma, dat reeds aangebracht werd door de pulpotomie nauwelijks en veroorzaakt geen circulatiestoornis door thrombusvorming.

In artikel II wordt het onderzoek beschreven naar het desinfecterend vermogen van de agentia met een lage formaldehyde concentratie bij bacterieel gecontamineerde, geëxponeerde pulpae. Bovendien werd ook de weefsel-compatibiliteit van de agentia bestudeerd.

Conclusie: De beide onderzochte desinfectantia waren effectief en werden goed verdragen door het pulpaweefsel.

In artikel III worden in vitro zowel als in vivo, enkele tandheelkundige cementen op hun biocompatibiliteit onderzocht.

Conclusie: Cavit blijkt een gunstiger overkappingsmateriaal

te zijn dan Nimeticap, terwijl Durelon, althans in vitro, even biocompatibel is als Cavit.

In artikel IV wordt een onderzoek beschreven naar een afsluiting van de geëxponeerde pulpa, die geen microlekkage van bacteriën vertoont. Een reden voor dit onderzoek was, dat tijdens de voorgaande experimenten veel van de overkapte geëxponeerde pulpae histologisch aantoonbaar positief waren voor bacteriën. Aangezien lekkage langs het afsluitmateriaal genoemd wordt als een van de oorzaken van ontstekingsreacties in het pulpaweefsel, was een bacterie-dichte afsluiting gewenst.

Conclusie: Afdichten van de geëxponeerde pulpa met Cavit en vervolgens sealen van de Cavit en het omringende glazuur, na etsen, met UV-polymerizerende Uvio-Bond, bleek bacterie-dicht af te sluiten, althans voor de duur van 42 dagen in dit dierexperiment.

In artikel V wordt het patroon van afgifte van het medicament Tantum uit verschillende tandheelkundige cementen beschreven. Dit in vitro experiment wordt uitgevoerd om inzicht te verkrijgen in de verwachte verschillen in uitlek uit hydrophiele en hydrophobe cementen. De resultaten geven aan welke materialen een zodanig afgiftepatroon van het antiphlogisticum vertonen dat dit kan bijdragen tot het verminderen van de ontstekings-reactie van de geëxponeerde pulpa.

Conclusie: Het verschil in afgiftepatroon dat Cavit en Durelon vertonen maakt beide geschikt om te gebruiken in een in vivo onderzoek naar de invloed van Tantum op de ontstekingsreactie in geëxponeerde, geïnfecteerde pulpae.

Om in vivo het effect te bestuderen van de uitlek van een antiphlogisticum uit tandheelkundige cementen als carrier van dit medicament, was het noodzakelijk te beschikken over een methode om experimenteel een pulpitis op te wekken. Deze methode wordt beschreven in artikel VI.

Conclusie: Na twee dagen heeft het appliceren van een waterige suspensie van 10^6/ml Strep. faecalis op de geëxponeerde pulpa gedurende 5 minuten een ontstekingsreactie tot gevolg, waarvan de ernst mild tot matig is.

Deze methode wordt gebruikt in het laatste onderzoek, dat in het artikel VII wordt beschreven. Hierin worden geëxponeerde pulpae, twee dagen nadat ze geïnfecteerd waren met Strep. faecalis, gedesinfecteerd en overkapt met cementen, die Tantum bevatten. De invloed van het antiphlogisticum Tantum op de ontstoken pulpae werd histologisch geëvalueerd.

Eindconclusie: Bij het overkappen van de geëxponeerde pulpa, is toediening van Tantum aan deze pulpa, via een gecontroleerde afgifte uit een carrier, effectief voor wat betreft het bevorderen van herstel van een gezonde conditie. Bovendien kan - zover het dierexperimenten betreft - een afsluiting verkregen worden, die bacterie-dicht is, door de caviteit af te sluiten met het biocompatibele Cavit en dit materiaal plus het omringende glazuur te verzegelen met Uvio-Bond.

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CIRCULATORY STASIS AND FIXATION OF PULP TISSUE AFTER PULPOTOMY USING AGENTS CONTAINING FORMALDEHYDE

A clinical experiment in monkeys

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ABSTRACT

After pulpotomy in monkey teeth, a 5 minute application of Formocresol, Alcoformol 19/60 or Alcoformol 8.75/60 produced a fixed area in the pulp adjacent to the wound surface. Apical to this surface, an area of autolysis was observed which in turn was followed by vital tissue. The presence of the intermediate area of autolysis suggested that the fixed tissue adjacent to the wound surface was caused by the medicament and not by the histological fixation.

Perfusion of the vascular system demonstrated the presence of thrombi.

Neither a drug fixed area, nor thrombi were observed in pulps after application of agents containing 0.25% - 4% formaldehyde and in the controls. In these cases, the zone of autolysis was found adjacent to the wound surface.

Based upon these findings it can be deduced that the high concentration of formaldehyde was the cause of the fixation and the thrombi. These agents can be considered to be more severe threats to the life of the dental pulp than agents low in formaldehyde.

INTRODUCTION

Tissue changes in the radicular pulp after Formocresol pulpotomy have been reported by many authors (Emmerson, Miyamoto, Sweet et al.[1], 1959; Mansukhani[2], 1959; Massler and Mansukhani[3], 1959; Dietz[4], 1961; Doyle[5], 1961; Doyle, McDonald and Mitchell[6], 1962; Berger[7,8], 1963, 1965;

Spedding[9], 1965; Spamer[10], 1965; Beaver[11], 1966; Harris[12], 1969; Mejàre, Hassalgren and Hammarström[13], 1976; for review see Berger[14], 1975). In these studies, routine histology was used[1-12] to detect the boundery between vital and non-vital pulp tissue with the exception of the study of Mejàre, et al.[13] who determined lactate dehydrogenase activity. Teeth from humans, from rhesus monkeys[9], deciduous [1-12] and permanent[2,3,13], and from rats[2,3] have been used. No major differences in pulp behaviour were observed[3] except in the case of rat pulps. Because of the aberrant behaviour of rat tissue, results obtained from this animal are not included in this paper.

The results of previous investigations can be classified according to the duration of application and the concentration of formaldehyde in the applied agents. In some cases the agent was applied for only 5 minutes and in other cases for a period of three days or longer. The concentration of formaldehyde varied from 19% to 4%. When these variables are combined, four sets of protocols can be differentiated.

1) Formocresol acting over extended period. In most routine histological investigations[2-10] 3 zones varying in length have been observed extending from the amputation site in an apical direction. The three zones generally consisted of (a) a zone of tissue with preserved cellular detail, (b) a zone with distinctly less cellular detail (pale staining tissue, coagulation necrosis) and (c) a zone of vital tissue, whether inflamed or not. Darker staining or enhanced eosinophilic staining of the layer of tissue adjacent to the wound surface, is often mentioned in these studies.

The well preserved zone was considered by many authors [2-5, 7-10] to be in vivo fixed by the medicament, whereas others[6,11] believed the zone with less cellular detail to be in vivo fixed as well.

Some investigators made conflicting observations. Beaver et al.[11] claimed to have found coagulation necrosis in the coronal third in 21 teeth, a zone with preserved cellular detail in only one tooth whereas two teeth demonstrated vital tissue up to the wound surface. Emmerson et al.[1] observed an eosinophilic zone which they considered to be drug fixed, whereas the remaining pulp was described as being vital.
Using enzyme histochemistry Mejàre et al.[12] found the whole pulp, except for an apical 3 mm, to be negative for lactate dehydrogenase and consequently to be affected by formaldehyde.

In general, the results of previous investigations demonstrated severe pulpal damage as evidenced by drug fixed tissue, areas of reduced cellular detail and enzyme negative zones. The cause of the pulpal damage was Formocresol acting over an extended period after pulpotomy.

2) Formocresol acting over 5 minutes. Three zones similar to the above mentioned zones were described by Spamer[10]. Massler and Mansukhani[3] who used a period of from 1-36 minutes, and Emmerson et al.[1] reported surface fixation only with normal tissue adjacent to it. Beaver et al.[11] observed that 12 out of 21 teeth had coagulation necrosis in the coronal third. Damage to the pulp is evident after this treatment although less well defined than after extended treatment.

3) Aqueous 4% formaldehyde solution acting over extended period. Mejàre et al.[13] were unable to delineate the penetration of formaldehyde by routine histology. Only an eosinophilic zone, adjacent to the wound surface, was found, which was followed by normal tissue. However, when applying lactate dehydrogenase histochemistry, a zone, adjacent to the amputation area, appeared to be negative. Although damage to the pulp is also evident in this case, a less radical affect seems to have taken place when compared with the extended Formocresol treatment.
4) Aqueous 4% formaldehyde solution acting over 5 minutes. No reports are known to the authors.

Dilated blood vessels containing aggregated erythrocytes have been mentioned by many authors after extended treatment[3,8,11], after 5 minutes treatment with Formocresol[12], and after 4% formaldehyde solution during an extended period[13]. The presence of such vessels might indicate thrombus formation.

The object of this investigation was to study the effects (circulatory stasis and other radicular pulp tissue reactions) of a reduction of the period of a single application to 5 minutes combined with reduction of the concentration of formaldehyde in the agent (4% or less).
For comparison, the same effects were studied after a single 5 minute application of agents containing formaldehyde in concentrations of 8.75% or 19%.

MATERIAL AND METHODS

Forty-one caries-free teeth, mainly premolars, of nine Rhesus monkey (Macaca mulatta) were used in this study. The teeth were scaled and radiographed some days before treatment.

The monkeys were anaesthetized using Nembutal, and the working field disinfected with Hibitane (0.5% w/v chlorhexidine digluconate in alcohol). Access to the pulp chambers was gained using a round bur and a Batt bur and the orifices of the root canals were slightly widened with a Gates Glidden drill. The pulp chambers were washed out by means of cotton pellets moistened with sterile physiological saline solution. After drying with sterile cotton pellets, 0.005 ml of one of the following solutions was applied to each pulp chamber by means of a microliter syringe: (i) Formocresol (FC) containing 19% formaldehyde* (Buckley's formula, Keur and Sneltjes, Haarlem, The Netherlands), (ii) Alcoformol 19/60 (AF 19/60) containing 19% formaldehyde dissolved in 60% ethanol, (iii) Alcoformol 8.75/60 (AF 8.75/60) containing 8.75% formaldehyde dissolved in 60% ethanol, (iv) aqueous solutions of 4%, 2%, 1%, 0.5%, 0.25% formaldehyde (F), (v) sterile physiological saline solution (control group). After 5 minutes application of each agent, the pulp chambers were dried by means of sterile cotton pellets. The access cavities were sealed with Cavit-W. Animals were sacrificed after experimental periods of 1 and 2 days.

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* Stock solution: 35% formaldehyde in water, analytical grade, catalogue no. 4003, Merck, Darmstadt, W. Germany.

To study tissue reactions, routine histological processing was preferred to histological processing after maceration of the tissue pieces, as the last mentioned kind of processing can indicate a drug fixed area (Simon and van Mullem[15]) but does not differentiate between in vivo autolysed tissue and vital tissue. To study thrombi, perfusion of the animals' circulatory system was performed previous to the histological fixation. Thus, the animals were sacrificed by perfusion, after Nembutal anaesthesia, first with physiological saline solution, then with a 2½% phosphate buffered glutaraldehyde solution. The pressure of perfusion was 84 mm Hg equal to the mean diastolic pressure in pentobarbital anaesthetized Rhesus monkeys (Hayden[16],1975). According to the authors' experience this pressure suffices to free normal oral blood vessels from cells. Parts of the jaws that contained the teeth were dissected and postfixed by immersion in the same fixative, decalcified and embedded in Paraplast. Mesio-distal 7 µm thick sections were HE, H only or Masson trichrome stained.

The root pulps were evaluated for a) areas adjacent to the wound surface containing preserved nuclei, b) areas containing nuclear degeneration and c) thrombi.

Nuclei were considered to be preserved when intact, haematoxylin positive and either with normal chromatin structure or homogeneously stained. Nuclear degeneration was considered to be present in cases where fragmented or vacuolar haematoxylin positive nuclei, eosinophilic nuclei, or no nuclei were observed. In such areas the tissue remnants, other than nuclei, were eosinophilic to varying degrees, smooth, finely granular or vacuolated.

Measurements of the length of areas were not made as in many teeth the coronal and the apical bounderies were observed in separate sections. Thrombi were scored using as criterion the presence of bloodcell filled vessels.

Statistical testing was performed using a version of Fisher's exact test of fourfold tables (testing was performed one-sided because the action of the formaldehyde could only be towards a preservation of the nuclei) and the test of Fisher as a test for independence. As level of significance $\alpha = 0.05$ was chosen.

RESULTS

Areas containing preserved nuclei, nuclear degeneration and thrombi were scored (Table). In areas containing preserved nuclei, differentiation could be made between the presence of preserved nuclei located at the entire wound surface (Fig. 1) and those located at the dentinal wall only. Scoring was performed separately for these locations. Preserved nuclei in the central stroma only were never observed.

Generally, nuclear preservation was not as good as normally seen in vital tissue fixed by standard histological techniques. Nuclei showing normal internal structure were seldom observed and only when using FC.

An area with preserved nuclei at the wound surface, when present, was always followed in apical direction, first by an autolysed area, then by an area of vital tissue whose morphology was preserved by the histological fixative.

Fig. 1. Photomicrograph of a Formocresol treated radicular pulp. A. Overall picture which demonstrates the darker stained cervical part and, in apical direction, the faintly stained part. B. Detail of darker part showing preserved nuclei (drug fixed area) C. Detail of faintly stained area which consists of autolysed tissue and thrombosed blood vessels containing some neutrophils. HE. x50 and x315.

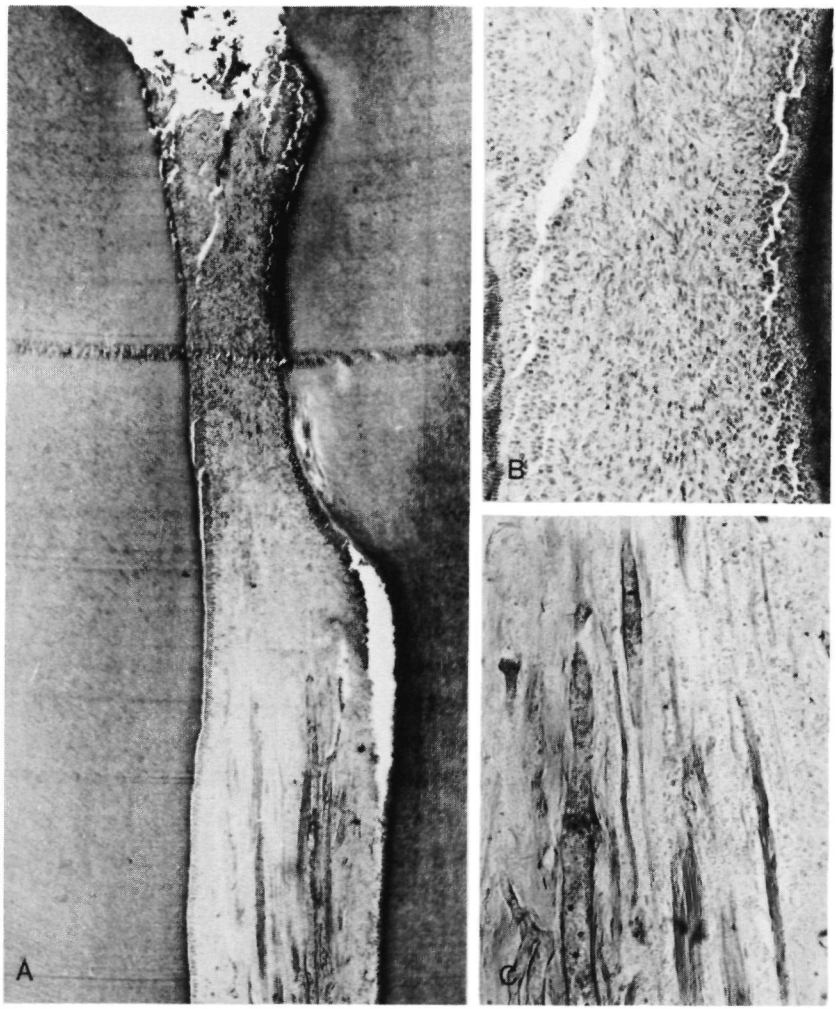


Table. Results of scoring nuclei and thrombi in the presence of several medicaments.

Agent	Sample size	Radicular pulps with preserved nuclei at wound surface		
		at entire wound surface	in tissue adjacent to dentinal wall only	no such area
FC	7	6	0	1
AF 19/60	8	6	0	2
AF 8.75/60	8	4	4	0
F 4%	6	0	0	6[1]
F 0.25% - 2%	4	0	1	3
Controls	6 + 2[2]	0	0	6

1) two teeth demonstrated a few faintly stained nuclei adjacent to the dentinal wall

2) vital pulp up to wound surface; no thrombi

Radicular pulps with preserved nuclei and thrombi in:				Radicular pulps not containing preserved nuclei and thrombi in:	
preserved area and autolysed area	preserved area only	auto-lysed area only	no thrombi	autolysed area	no thrombi
6	0	0	0	0	1
4	0	1	1	2	0
7	0	1	0	0	0
0	0	0	0	0	6
0	1	0	0	0	3
0	0	0	0	0	6

A number of teeth demonstrated no area with preserved nuclei at the wound surface (Table). Instead, the autolysed tissue at the amputation site (Fig. 2) was followed apically by vital tissue. In two teeth of the control group preserved pulpal nuclei were observed extending from the apical foramen to the wound surface. The quality of the tissue was such that in the living animals, these pulps must have been vital and thus were fixed by the histological fixation.

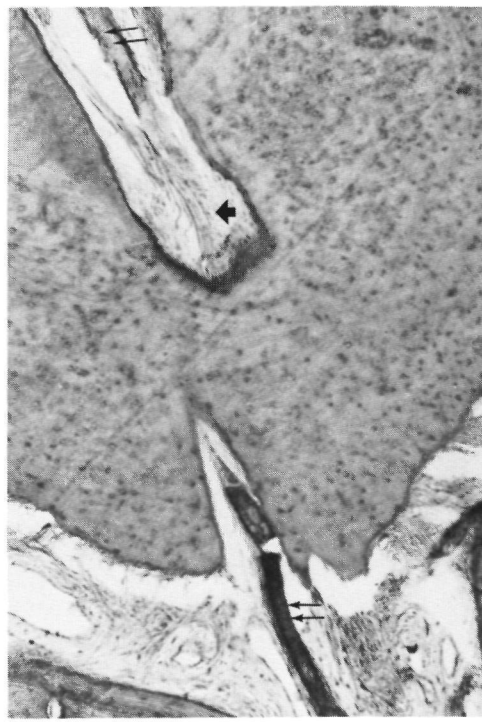
Table also represents the observations on thrombi which were seen in areas with preserved nuclei and/or in autolysed areas (Fig. 1). An exceptional case is shown in Fig.3 where a thrombus is seen in the vital apical periodontium. In the remaining teeth, all blood vessels in the periodontal and other tissues had been cleared of erythrocytes by the perfusion of the vascular system. Masson trichrome staining of sections did not demonstrate a red stain in the cut dentine, indicating that no burn was caused during drilling.

Statistical testing, by means of 2 x 2 contingency tables, of the presence (20 teeth) and the absence (3 teeth) of an area with preserved nuclei, using agents with formaldehyde concentrations of 8.75% or higher, versus the presence (0 teeth) and absence (8 teeth) in the controls revealed that the teeth treated with the agents had more areas with preserved nuclei ($p << 0.001$). A similar comparison of the scores obtained after the use of agents against formaldehyde concentrations of 4% and lower versus the scores in the controls produced a p-value of 0.556.
Testing the presence (21 teeth) and the absence (2 teeth) of thrombi using agents high in formaldehyde, versus pre-

sence (0 teeth) and absence (8 teeth) in the controls demonstrated that teeth treated with the agents had more thrombi ($p << 0.001$). When comparing the scores for thrombi which were obtained after the use of agents low in formaldehyde versus the controls a p-value of 0.556 was found. The test of Fisher for independence of the occurrence of an area containing preserved nuclei and the occurrence of thrombi, in the areas with preserved nuclei and/or in the autolysed areas revealed $p << 0.001$, independence being rejected.

Fig. 2. Photomicrograph of a control tooth. Autolysed tissue is observed in the part of the pulp shown here. No thrombi. Debris at wound surface. HE. x55.

Fig. 3. An exceptional case of thrombus formation in a AF 8.75/60 treated radicular pulp: the pulpal blood vessel is filled with blood cells (thin arrows) even in the periapical periodontium. All other periodontal vessels were emptied by the perfusion of the animal's vascular system. The most apical part of the pulp was vital (thick arrow). HE. x85.



Pulp tissue reaction to agents with high concentrations of formaldehyde (FC, AF 19/60 and AF 8.75/60)

In 20 out of 23 teeth an area of radicular pulp tissue was observed at the wound surface which demonstrated a better preservation of nuclei than the adjacent autolysed areas. This was different from the controls ($p << 0.001$) where no preserved areas were found. The quality of the preservation was not as good as was observed by Simon and van Mullem[15] after application of pastes containing paraformaldehyde for experimental periods of seven and 14 days. They attributed the nuclear preservation to a fixing capacity of the drugs. Although in the present material the nuclei in the superficial tissue were somewhat less preserved, it still seems impossible that such tissue would remain isolated above autolytic tissue unless fixation in vivo had occurred. This observation indicated that the application of agents with a high concentration of formaldehyde, applied for five minutes only, resulted in a drug fixed area. Of these agents AF 8.75/60 appeared to be the least powerful in causing a drug fixed area, as 50% of the case demonstrated preserved nuclei adjacent to the dentinal wall only (Table). It also appeared that the in vivo effect of formaldehyde on pulp tissue could be studied by routine histological techniques in spite of the fact that the tissue was exposed to a fixative in vivo as well as at the start of the histological processing. This was due to the presence of a zone of autolysis which histologically separated the drug fixed zone from the apical vital tissue.

The above mentioned finding of three zones corroborate the

findings of Mansukhani[2], 1959; Massler and Mansukhani[3], 1959; Dietz[4], 1961; Doyle[5], 1961; Doyle, McDonald and Mitchell[6], 1962; Berger[7,8], 1963, 1965; Spedding[9], 1963; and Spamer[10], 1965. However, the present observations were made after a single 5 minute application of FC, whereas most of the above mentioned authors[2-10] obtained the three zones after application over an extended period, i.e. a 5 minute application followed by the action of FC which had been applied to a cotton pellet, or paper point, or added to a ZnO paste or a ZnOE base acting over a period of at least three days.

Of those investigators who studied the reactions after a 5 minute application, only Spamer[10] observed three zones. Emmerson et al.[1] and Massler and Mansukhani[3] described an eosinophilic zone only, while Beaver[11] described coagulation necrosis or inflammatory reactions. The discrepancy between our findings and those of others[1,3,11] might be explained by the orifices of the root canals being slightly widened in the present study. The wider entrance may have facilitated the penetration of the agents.

Pulp tissue reaction to agents with 4% or less formaldehyde

In 9 out of 10 cases, the teeth which were treated with agents low in formaldehyde (0.25% - 4%), did not demonstrate a drug fixed area, but did demonstrate an autolysed zone at the wound surface, vital tissue being located apically in all cases. In the remaining tooth, preserved nuclei were observed at the dentinal wall only. Thus, in this experiment where the orifice of the root canal was slightly widened, to promote the penetration of agents, even F 4% showed insufficient capacity to keep nuclei from autolysis. This conclusion was not contradicted by the results of sta-

tistical testing of these groups versus the controls.

The observed autolysed area combined with the drug fixed area, if present, may well agree with the lactate dehydrogenase negative zone which was demonstrated by Mejäre, Hassalgren and Hammarström[13]. The length of this zone (3 - 5 mm) was dependent on the experimental period which was 1, 4, 8 or 16 days.

The autolysis observed in the control group was considered to be evoked by the operative trauma. In the teeth treated with an agent low in formaldehyde, the autolysed area might have been provoked by the combination of mechanical and chemical trauma. In these cases where an agent high in formaldehyde was used, the stronger toxic effect was represented by the drug fixed area at the side where the concentration of formaldehyde in the pulp tissue must have been highest: i.e. adjacent to the amputation site. Damage caused by heat generated by drilling can be excluded on the basis of the Masson trichrome staining results.

Vascular changes

Circulatory stasis could be studied in the present material because blood vessels in which circulation was possible in vivo, were cleared of blood cells during perfusion of the animal, first with physiological saline solution, then with the histological fixative. Each blood vessel filled with erythrocytes was part of a thrombus and thus indicated circulatory stasis.

Thrombi were not observed in the controls. Twenty-one out of 23 teeth which received agents with a high concentra-

tion of formaldehyde (FC, AF 19/60 and AF 8.75/60) demonstrated thrombi. The pooled results of these agents was statistically significant ($p << 0.001$) compared with the controls. In a few cases the thrombi were located either in the drug fixed area or in the autolysed area, in all other cases they were observed in both areas. Formaldehyde, being the common factor in these agents, appeared to be responsible for the thrombi. Agents low in formaldehyde (F 0.25% - 4%) did not provoke thrombi, with the exception of 1 tooth in 10 and this result was not significantly different from the controls.

It can be concluded that, under the conditions of this experiment, agents with concentrations of formaldehyde of 8.75% and higher are able to provoke haemostasis, whereas agents with lower concentrations are not powerful enough to do so.

Mutal relation between pulp tissue reaction and vascular changes

The positive correlation of drug fixed areas with thrombi is evident from the results (Table). Moreover, the significant rejection of independence of the two phenomena in Fisher's test for independence is indicative of correlation. It may be interpreted in such a way that a high formaldehyde concentration is the factor provoking both the drug fixed areas and the thrombi. In addition, it is conceivable that the area of autolysed pulp tissue, in the presence of a thrombosed blood vessel, is ischaemic. This lack of oxygen might add to the appearance of autolysis. Moreover, a thrombus might enhance the penetration of formaldehyde molecules into the radicular pulp tissue since

they are not carried away by the blood from the affected area because of the circulatory stasis. Thus, at a given point in the tissue, the concentration of formaldehyde may be higher in the presence of stasis than without stasis. This may lead to further thrombus formation. This self-propagating process of thrombus formation ultimately might fade out because of dilution of the formaldehyde in the pulp tissue and chemical bonding to tissue components. Using agents low in formaldehyde, concentrations too low to provoke thrombi might be present in the superficial layers of the tissue right from the start. This is supported by the fact that drug fixed areas and thrombi were not observed in these groups of teeth.

Conclusion

A 5 minute application of aqueous solutions of formaldehyde in concentrations of 0.25% - 4% appeared to be less toxic to the remaining pulp after pulpotomy than Formocresol and other agents high in formaldehyde.

If the findings of this animal study can be extrapolated to the human situation after Formocresol pulpotomy, both circulatory stasis and tissue fixation can be considered to be threats to the life of the radicular pulp. These phenomena might well contribute to the relatively high percentage of failures after Formocresol pulpotomy in deciduous teeth.

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THE EFFECTIVENESS OF TWO DISINFECTANTS AND THEIR ACTION ON THE EXPOSED PULP

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ABSTRACT

Anti-microbial effectiveness of two agents. Alcoformol 1/10 and 3/20 were studied in vitro and in vivo. Both agents were equally effective and compatible with pulpal tissues. Comparison of the in vitro and the in vivo results indicated the in vitro study to be a well predictive test.

INTRODUCTION

The reaction of radicular pulp tissue after pulpotomy followed by the application of Formocresol has been extensively investigated (e.g. Massler and Mansukhani[1], 1959; Doyle[2], 1962 and Berger[3,4], 1965, 1972). Most authors agreed on the tissue changes when Formocresol was applied for at least three days.

These changes consisted of three zones in apical direction:

1. A drug fixed zone at the amputation site.
2. A zone of autolysed tissue.
3. Vital radicular pulp tissue.

When Formocresol was applied for five minutes only, the observed tissue changes seemed to be less uniform. Beaver[5] (1966) reported coagulation necrosis or vital tissue at the wound surface, whereas Massler and Mansukhani[1] reported an eosinophilic zone at the amputation site which they considered to be fixed by the medicament. Spamer[6] (1972) and van Mullem and Wijnbergen[7] (1982) observed three zones similar to those described above. However, after a five minutes' application of agents containing formaldehyde in concentrations lower than in Formocresol, i.e. ¼% - 4% aqueous formaldehyde solutions, van Mullem and Wijnbergen[7] observed a zone of autolysed tissue adjacent to the ampu-

tation site in the experimental teeth which was similar to the controls. They stated that after the use of these solutions no toxic effect could be observed to be added to that of the operative trauma. They came to the conclusion that short term application combined with decreased formaldehyde concentrations reduced pulp tissue changes.

The question arised whether agents with low concentrations of formaldehyde are effective as disinfectants. In addition, it might be questioned whether pulp amputation is a necessary step after exposure. Healing potential might still be present in an exposed pulp and ultimately might lead to recovery without amputation.

The aim of this combined in vivo and in vitro study was to investigate, after pulp exposure without pulpotomy, the anti-microbial effectiveness and pulp tissue reaction to two Alcoformol (AF) agents containing 1% and 3% formaldehyde after a five minute application.

MATERIAL AND METHODS

To study the disinfecting power of Alcoformol 1/10[x)] and Alcoformol 3/20[+)] in vitro, the root canal and pulp chambers of 43 extracted human teeth, which were previously

x) AF 1/10: 0.6 ml of an aqueous 35% formaldehyde solution (Merck, Darmstadt, Germany, analytical grade, catalog no. 4003) and 2 ml ethyl alcohol 100% were added to 17.4 ml of water.

+) AF 3/20: 1.8 ml of a 35% formaldehyde solution and 4 ml alcohol 100% were added to 14.2 ml of water.

stored in water, were retrograde widened using Torpan reamers of a diameter of 1.8 mm. Each tooth was attached to the bottom of a sterile Petri dish with Kerr impression compound, then sterilized with acetone and dried in a stream of sterile air. They were stored overnight at 37°C in the Petri dishes containing moisturized air. The canals and chambers were filled with sterile Oxoid Brain Heart Infusion agar medium and stored at 4°C for another night. The next day a buccal cavity was drilled in each tooth with a sterile 1 mm Ø spiral bur, exposing the agar in the pulp chamber. All cavities were filled with a freshly prepared aqueous 10^6/ml suspension of Strep. faecalis by means of a 1 ml syringe. After 5 minutes the cavities were dried with sterile paper points, then cultures were taken by the method described below to demonstrate the vitality of the bacteria in the cavities.
Another infection with the same suspension of bacteria was carried out during 3 minutes. After drying the cavities disinfection for a period of 5 minutes was performed using 0.005 ml of one of the following solutions (the number of teeth in each sample is given in parenthesis): none (9), sterile physiological saline solution (10), AF 1/10 (12), AF 3/20 (12). Subsequently, the cavities were dried with sterile paper points and sealed with gutta-percha points. The Petri dishes containing the teeth were stored at 37°C for 3 days. Then two cultures were taken from each cavity by means of sterile paper points moistened with saline solution. They were cultures in Brain Heart Infusion both at 37°C. The tubes were read after 3 days.

To study in vivo the anti-microbial effectiveness of AF 1/10 and AF 3/20 and the tissue reaction to these prescriptions, exposures were made in 55 deciduous anterior teeth

of Macaca arctoïdes.

The operating field was desinfected with Hibitane (0.5% chlorhexidine digluconate in alcohol) and a glass bead sterilizer was used to disinfect the instruments. The drilling of the 1 mm Ø cavity and the exposure of the pulp was performed by means of an apparatus with which standardized exposures could be achieved (The and van Mullem[8]). The experiment comprised 5 groups of teeth (the sample size is given in parenthesis):

A. non-infected controls (22),
B. infected controls (5),
C. infected and AF 1/10-treated teeth (14),
D. infected and AF 3/20-treated teeth (9), and
E. teeth that were processed shortly after exposure (5).

The last mentioned group was used to study the initial effect of the operative trauma.

Infection was carried out using an aqueous suspension of 10^6/ml Strep. faecalis. After 5 minutes the cavities were dried with sterile paper points and the teeth of group C and D received 0.005 ml of AF 1/10 or AF 3/20, resp. All teeth were sealed with Cavit[x)]. The Cavit and the marginal enamel were covered, after etching, with Concise bond[+)]. The experimental period was 14 days for the groups A, C and D. For group B the experimental period was limited to 7 days for ethical reasons. The teeth were extracted and fixed in neutral 4% formaldehyde solution, after removal of the apical third with a diamond disk. After decalcification

x) ESPE, Seefeld/Oberbay., W. Germany.
+) 3M, St. Paul, Minnesota, U.S.A.

and embedding in Paraplast, 7 µm thick bucco-lingual sections, in which the cavities appeared longitudinally, were haematoxylin-eosin stained.

The presence or absence of bacteria was scored using Brown and Brenn stained sections. Evaluation of the pulp tissue reactions was performed in the teeth which were Brown and Brenn negative, to exclude reaction of bacterial origin.

Statistical testing was performed using the test of Kruskall and Wallis, the test for trend of van Eeden and an exact version of Fisher's test for fourfold tables. This test was applied one-sidedly based on the expectation that the dental procedures would tend to reduce the number of bacteria, or would add an extra noxious effect to that of the operative trauma. The level of significance (α) was fixed at 0.05.

RESULTS

In vitro experiment

The results of culturing from the previously infected teeth which received no treatment (controls), physiological saline solution, AF 1/10 or AF 3/20 are presented in Table 1. The results of statistical analysis of these data are given in Table 2. A strongly significant trend towards more negative teeth in the groups which were treated with AF, is found. Analysis of this trend revealed no significances, when the controls were compared with the physiological saline treated group and when both AF treated groups were compared. The trend is explained by the significant difference between the physiological saline treated group and

Table 1. Results of culturing from 43 previously infected teeth in the in vitro experiment after a 3-day experimental period.

agent / culture result	none (controls)	phys. saline	AF 1/10	AF 3/20
+	9	7	3	2
-	0	3	9	10
sample size	9	10	12	12

Table 2. p-Values of one-sided statistical test performed on the data in Table 1.

test of van Eeden on series of groups			p =	<<0.001 ↑
2 x 2 contingency tables				
controls	vs	phys. saline	p =	0.124
phys. saline	vs	AF 1/10	p =	0.046 ↑
AF 1/10	vs	AF 3/20	p =	0.500
pooled controls and phys. saline vs pooled AF 1/10 and AF 3/20			p =	<<0.001 ↑

↑: last mentioned group contains significantly more negative teeth than first group

the AF 1/10 group. This is supported strongly significantly by the comparison of the pool of the controls and the saline group with the pool of both groups treated with AF.

In vivo experiment

The results of scoring presence or absence of bacteria after Brown and Brenn staining of slides from the teeth of the 4 experimental groups are represented in Table 3. Statistical comparison of these results are given in Table 4. Comparison of the in vivo results with those obtained in vitro for the AF 1/10 treated group, the AF 3/20 group and for the pool of both groups revealed p-values of 1.000, 0.611 and 0.740, resp.

Tissue reactions were studied in those teeth of groups A, C and D which were Brown and Brenn negative and in the teeth of group B (the infected, Brown and Brenn positive controls). In addition, the effect of the operative trauma was studied in the teeth which were extracted shortly after exposure. In these teeth, a reduction of the odontoblast layer in the area of the cut dentinal tubules was observed, together with aspiration of odontoblast nuclei (Fig. 1). In most cases a dilatation of the pulpal capillaries was found.

In groups A, C and D, after 14 days, vaso-dilatation was still present, while aspiration of odontoblast nuclei could not be observed. At the site of the initially reduced layer of odontoblasts, a repaired layer of hard tissue forming cells was found in most cases.

Masson trichrome staining did not reveal a red stain in any of the cut dentinal walls.

The results of scoring inflammatory reaction and deposition of reactive dentine are represented in Table 5.
The test of Kruskall and Wallis did not reveal statistically significant differences in level between the groups A, C and D, nor for the inflammatory reaction, nor for the deposition of reactive dentine.

Table 3. Results of scoring bacteria in teeth in the in vivo experiment as observed after Brown and Brenn staining and an experimental period of 14 days (7 days in group B).

group / score	A non-infected controls	B infected controls	C infected and AF 1/10 treated teeth	D infected and AF 3/20 treated teeth
+	5	5	3	3
-	17	0	11	6
sample size	22	5	14	9

Table 4. p-Values of one-sided statistical tests performed on the bacteriological data of the groups mentioned in Table 3.

trend test of van Eeden on groups A, C and D	p = 0.268
trend test of van Eeden on groups B, C and D	p = 0.008↑
2 x 2 contingency tables:	
controls (B) vs AF 1/10 (C)	p = 0.005↑
controls (B) vs AF 3/20 (D)	p = 0.028↑
AF 1/10 (C) vs AF 3/20 (D)	p = 0.868

↑ last mentioned group contains significantly more negative teeth than first mentioned group.

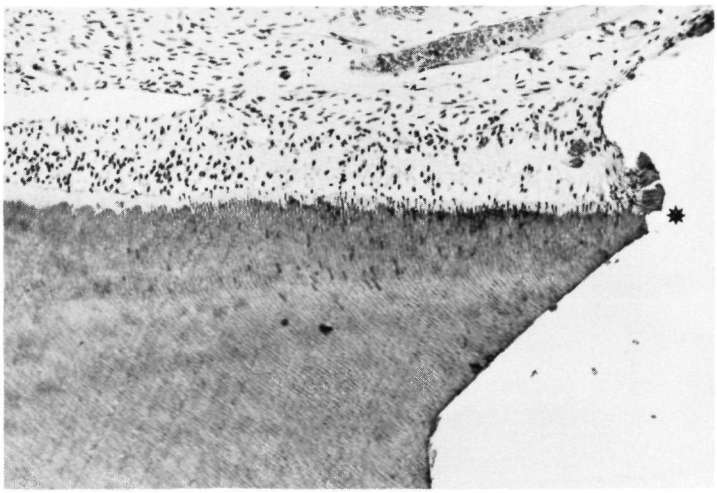
Table 5. Results of scoring inflammatory reaction and deposition of reactive dentine in Brown and Brenn negative teeth of groups A, C and D.

	inflammatory reaction[1]			deposition react. dentine[2]		
groups	A	B	D	A	C	D
score	non-infected controls	AF 1/10	AF 3/20	non-infected controls	AF 1/10	AF 3/20
-	8	5	3	4	1	0
±	4	1	2	6	6	2
+	3	3	1	7	4	3
++	2	2	0	0	0	0
n =	17	11	6	17	11	5

1) - = no reaction observed or very few inflammatory cells,
± = scattered inflammatory cells near wound surface,
+ = a focus or band of inflammatory cells near wound surface,
++ = strongly degenerative tissue changes or abscess formation,

2) - = no reactive dentine formation,
± = thin layer of intensely eosinophil dentinoid adjacent to exposure; presence of active, but not high cylindrical, hard tissue forming cells,
+ = thick layer of eosinophil dentinoid, with or without enclosed cells, with or without high cylindrical hard tissue forming cells.

Fig. 1. Photomicrograph of a tooth extracted shortly after pulp exposure (✱) and representing the tip of the bur. Initial tissue changes, aspiration of odontoblast nuclei and reduction of the odontoblast layer in the area of the cut dentinal tubuli can be seen. HE. x40.



The in vitro experiment was designed to shed some light on the question whether disinfection with agents containing formaldehyde in concentrations as low as 1% or 3% were effective under the conditions of in vivo application.

The presence of ethyl alcohol in the Alcoformol agents served to lower their surface tensions and to improve their wetting properties. A group of teeth treated with physiological saline solution was included to study whether the results obtained with the AF-agents could be interpreted as a mere rinsing effect (Table 1). The significance which was obtained, comparing the results of the physiological saline solution treated group with the group of teeth treated with AF 1/10 indicated that rinsing was not the only explanation for the better results obtained with AF 1/10 (Table 2). The strongly significant trend towards more negative teeth in the AF treated groups and the strong significance obtained when comparing both pools, indicated AF 1/10 and AF 3/20 to exert disinfecting power. However, a significant difference between the disinfecting power of AF 1/10 and AF 3/20 was not found. The number of teeth that remained positive at the end of the experiment in both AF treated groups (2 and 3; Table 1), can be explained by taking the conditions of the experiment into account. I.e. the narrow cylindrical cavity in which an air bubble might have prevented the agent from contacting all infected surfaces, notwithstanding its favourable physical properties. The shape of the cavities in this investigation was distinctly different from the shape of cavities after exposure normally seen in clinical practice which are wider and thus more easily accessible. Therefore disinfection would be

more complete in these situations.

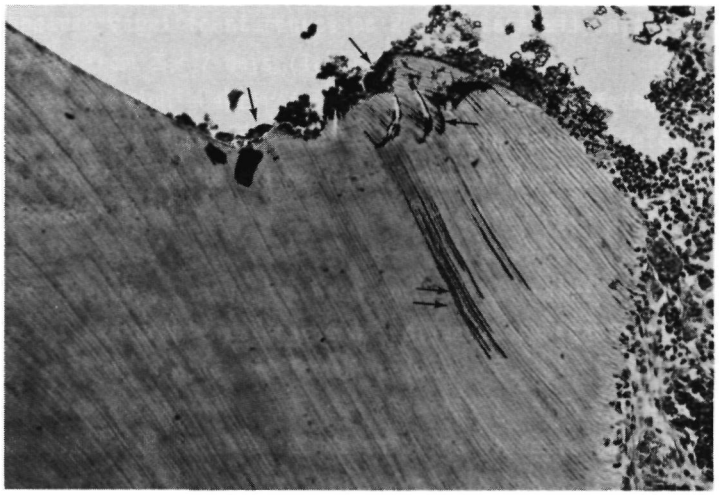
Thus it can be concluded from this in vitro experiment that both AF 1/10 and AF 3/20 can be considered effective disinfectants.

In the in vivo experiment both AF 1/10 and 3/20 appeared after an experimental period of 14 days to be effective disinfectants when compared with the infected controls (Table 4). The experimental period of the infected controls was limited to 7 days in order to limit the animals' suffering from pulpitis of bacterial origin. Moreover, it seemed unlikely to us that bacterial contamination, if manifest after 7 days, would be much reduced in another 7 days. For these reasons, the differences in duration of the experimental period were disregarded in the above comparison.

The 3 positive teeth (Fig. 2) in each of the groups C and D can be explained in a similar way as the positive teeth after infection and disinfection in the in vitro experiment. In addition, in this experiment microleakage past the temporary filling material is also a possible explanation. It can be concluded that both AF 1/10 and AF 3/20 are effective disinfectants under the conditions of this in vivo experiment.

A statistical comparison of the bacteriological results obtained in the in vivo experiment with those of the in vitro study was made. From the non-significances which were revealed, it is tempting to conclude that the results after 3 days in the in vitro experiment, were all predictive of the results which were obtained in dog teeth after 14 days.

Fig. 2. Detail of a cavity wall of a AF 1/10 treated tooth which remained positive for bacteria. At the wall, in cracks and in dentinal tubuli (arrows), Brown and Brenn stained microbes can be observed. x50.

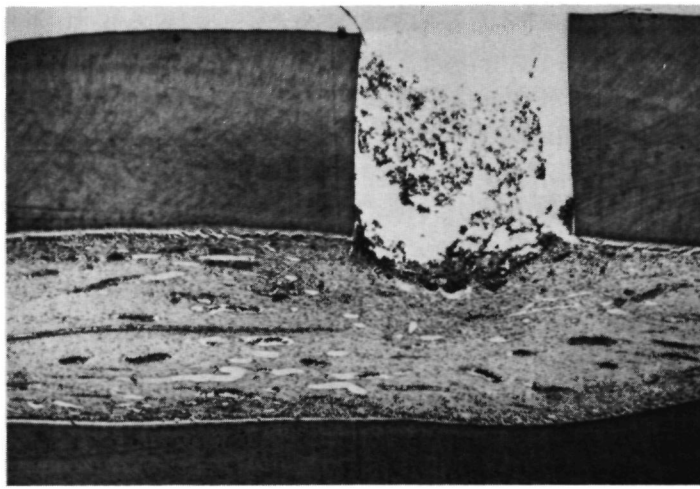


With regard to tissue changes due to the operative procedures no burn was caused as demonstrated by the Masson staining results. The tissue changes which were observed immediately after exposure (Fig. 1) appeared to be greatly reduced after 14 days. Autolytic areas, as observed by van Mullem and Wijnbergen[7] in pulpotomized teeth of which the orifices of the root canals were widened, were not observed in this study where the pulps were exposed only. Here the pulps were vital both in the controls and experimental groups. The absence of autolytic areas may be explained by the much smaller mechanical trauma in this study.

Regarding inflammatory reaction in the pulp and deposition of reactive dentine adjacent to the cut dentinal tubules (Fig. 3) no differences were found when comparing the non-infected controls with either the AF 1/10 or AF 3/20 treated groups.

The overall-conclusion from this study is that 1% or 3% formaldehyde in ethyl alcohol (AF 1/10 and AF 3/20) exerts sufficient anti-microbial power, both are equally effective and are well tolerated by the exposed pulp.

Fig. 3. AF 1/10. Photomicrograph of an exposed, infected and disinfected tooth which was negative for microbes as observed after Brown and Brenn staining. Dilated blood vessels and concentration of round cells near pulp wound are indicative of an inflammatory reaction grade+. HE. x12.5.



SUMMARY

The anti-microbial effectiveness of AF 1/10 and AF 3/20 (1% or 3% formaldehyde in 10% or 20% ethyl alcohol) was studied in single rooted teeth in vitro and in vivo. In the in vitro study, the agar medium in the pulp chambers of 43 extracted human teeth was infected after exposure with Strep. faecalis. After 3 days, the results of culturing from the cavities revealed AF 1/10 and AF 3/20 to be equally effective disinfectants and to be more effective than mere rinsing of the cavity with a physiological saline solution. The results after 14 days of the in vivo experiment in 28 deciduous anterior monkey teeth supported the in vitro investigation. From a comparison of the results of both studies, the in vitro experiment appeared to possess a highly predictive value. The results of the in vivo experiment revealed that 1) the initial effect of the operative trauma was much reduced after 14 days and 2) with regard to inflammatory reaction and deposition of reactive dentine, AF 1/10 and AF 3/20 did not add an appreciable toxic effect to that of the operative trauma. (n = 34).

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CAPPING OF EXPOSED PULPS WITH CAVIT-W OR NIMETICAP

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INTRODUCTION

In the case a drug has to be administrated locally after pulp exposure, for a period of time larger than a 5-minutes' application, a drug dissipating carrier is necessary. Since promotion of healing the dental pulp is the aim of such drug administration, cytotoxicity of the carrier per se can have influence on the rate of the healing process.

In the presence of an exposure, restoration of mechanical strength is another desideratum.
Commercially available filling materials might act as a carrier and can restore mechanical strength. Given a drug, the hydrophilicity of the filling material will determine a.o. the release of the drug from it: in a pilot-study it was shown that a readily water-soluble drug was dissipated to a buffer solution at a higher rate from a carrier with a distinct water economy than from a hydrophobic filling material.

Therefore, the aim of this investigation was to study cytotoxicity of two hydrophilic filling materials (Cavit-W and Durelon) and of two hydrophobic materials (Concise-paste and Nimeticap).
There were two parts in this investigation: an in vitro study, comprising the filling materials of both groups, and an in vivo study comrpising that material of each group which would appear the least cytotoxic in the in vitro investigation. In any case the results of sealing with Cavit-W would be added since it was the sealing material used after pulp exposure in previous studies (Van Mullem and Wijnbergen[1] an Wijnbergen and Van Mullem[2]).

MATERIAL AND METHODS

To study the in vitro biocompatability of Cavit-W[+], Concise[x], Durelon[+], 3 manufactures of Nimeticap[+], (commercially obtained old and new formula and remanufactured old formula which was specially made for us under the original specifications) and ZnO-eugenol[0] the agar overlay technique of Guess, Rosenbluth, Smidt et al.[3] (1965) was used.

Plastic Petri dishes (100 mm in diameter) filled with 10 ml Ham F 10 medium (Difco) containing 15% fetal calf serum, 100 units potassium penicillin G and 100 µg streptomycin sulfate were seeded with 1.5 x 10^6 cells of a human fibroblast strain. After incubation during 48 hours at 37°C in an atmosphere of 5% carbon dioxide in air, the cells were washed with a phosphate buffered saline solution, then gently covered with a still liquid (about 48°C) medium containing 1% calf serum and 1% agar. After solidification the cell cultures were vitally stained by pipetting 10 ml of a 0.01% neutral red solution on the surface of the agar. After 15 minutes the staining solution was removed by aspiration.

The materials to be tested were prepared according to manufacturers instructions and teflon rings (inner diameter 10 mm, outer diameter 13 mm and 1.5 mm in thickness) were fil-

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+) ESPE, Seefeld/Oberbay., W. Germany

x) 3M, St. Paul, Minnesota, U.S.A.

0) Dental Mfg. Company v/h Kraepelien & Holm, Bussum, Netherlands

led with the materials. Four such samples, whether freshly mixed or immediately after solidification, were applied to the surface of the agar overlay. In each Petri dish one of these rings always was filled with solidified ZnO-eugenol which was used as the reference material.

After 24 hours, for each ring the boundery between stained and unstained cells was marked with the aid of an inverted microscope and the diameter of the unstained area was measured in two directions which were perpendicular to each other. The radius was calculated and expressed as percentage of the ZnO-eugenol reference.

To investigate Nimeticap and Cavit under clinical conditions exposures were made in teeth of two animal species: monkeys (Macaca arctoïdes) and beagle dogs. This was because of the limited availability of monkeys. Thirty eight deciduous teeth from 9-month old monkeys and 24 permanent teeth from 8-month old dogs were used.
The operating field was disinfected with 0.5 chlorhexidine digluconate in alcohol. A glass bead sterilizer was used to disinfect the instruments. The drilling of the 1 mm Ø cavity and the exposure of the pulp was performed by means of an apparatus with which standardized exposures could be achieved (The and van Mullem[4]). After exposure the cavities were sealed with Nimeticap remanufactured old formula (40) or Cavit (22). The marginal enamel and the filling materials were covered with Concise bonding or Estilux after etching. The experimental period was 14 days. The teeth were extracted and fixed in neutral 4% formaldehyde solution, after removing the apical third of the root with a diamond disk. Decalcified, 7 µm thick Paraplast sections, in which the cavities and the pulpae appeared longitudi-

nally, were haematoxylin-eosin or Brown and Brenn stained. For evaluation of the pulp tissue reaction to the capping material only teeth that demonstrated to be Brown and Brenn negative were used to exclude reaction of bacterial origin.

Statistical testing was performed using the Mann and Whitney U-test (Wilcoxon's test) or an exact version of Fisher 's test for fourfold tables. As level of significance $\alpha = 0.05$ was chosen.

RESULTS

In vitro experiment

The results of testing the filling materials under investigation, using an agar-overlay technique and human skin fibroblasts, are represented in Fig. 1. The materials were applied either freshly mixed or after solidification. Pairwise statistical testing of the results thus obtained revealed no significances (Table 1). The remaining statistical tests were performed using the results after solidification of the materials.

The results of comparing cytotoxicity of the materials mutually, are given in Table 2. There appeared to be strongly significant differences between the three formulas of Nimeticap. However, even the worst of the three (Nimeticap new formula) demonstrated to be significantly less cytotoxic when compared with Durelon, Cavit and Concise-paste. Within the group of the last mentioned materials no significant differences were revealed.
ZnO-eugenol (the positive control) appeared to differ

(strongly) significantly from Durelon, Concise-paste and Cavit.

In addition to the above results of measuring area-diameters, the occurrence of a precipitate in the agar-layer under the pellets consisting of Cavit can be mentioned.

Experiment under clinical conditions in animal teeth

After exposure and an experimental period of 14 days, the presence of bacteria in teeth at the wound surface and/or in the cavity was scored using Brown and Brenn stained tissue sections.
The scoring results, for each of the 3 groups to which the teeth belonged, are represented in Table 3.

Pulp tissue reaction to the material which was used to seal the cavity and which was in direct contact with the pulp wound was studied in the teeth which were negative for bacteria. The results are given in Table 4.

Statistical comparison of the results of monkey teeth with those of dogs, both sealed with Nimeticap, could be performed using a fourfold table in which acceptable tissue reactions (- and $\pm$) were distinguished from non-acceptable reactions (+ and ++). A two-sided p-value of 1.000 was revealed. In this case a Mann and Whitney U-test was not applicable because of a tie in the + class (Table 4). When the results for Nimeticap in dog and monkey teeth were pooled and tested against the results after sealing with Cavit, a two-sided p-value of 0.008 was found. Cavit appeared in vivo to be less cytotoxic than Nimeticap, remanufactured old formula.

Fig. 1. In vitro cytotoxicity of 6 filling materials relative to the standard (100%): freshly mixed ZnO-eugenol. Dark column: applied after solidification, light column: freshly mixed.
Sample size is given on top of each column.

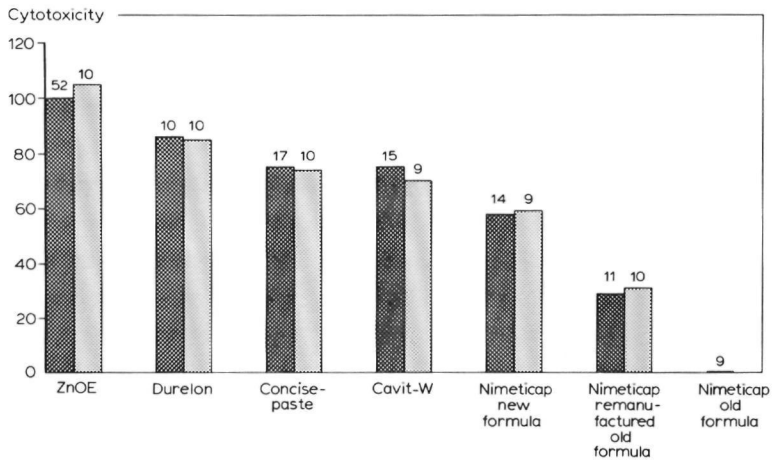

Table 1. Two-sided p-values of Mann and Whitney U-tests on in vitro results obtained after application of the material freshly mixed versus after solidification.

Filling material	p-value
Nimeticap remanufactured old formula	0.772
Nimeticap new formula	0.711
Concise paste	0.603
Durelon	0.912
Cavit-W	0.308
ZnOE	1.000

Results of freshly mixed Nimeticap old formula are not present because the material was no longer available.

Table 2. Two-sided p-values of Mann and Whitney U-tests on in vitro results obtained with seven filling materials after solidification.

Filling materials			p-values
Nimeticap old formula	vs	Nimeticap remanufactured old formula	<< 0.001↑[1)]
Nimeticap remanufactured old formula	vs	Nimeticap new formula	<< 0.001↑
Nimeticap remanufactured old formula	vs	Durelon	<< 0.001↑
	vs	Cavit-W	<< 0.001↑
	vs	Concise-paste	<< 0.001↑
Cavit-W	vs	Concise-paste	0.764
Concise-paste	vs	Durelon	0.384
Cavit-W	vs	Durelon	0.117
Durelon	vs	ZnOE	0.004↑
Concise-paste	vs	ZnOE	0.012↑
Cavit-W	vs	ZnOE	0.001↑

↑ last mentioned material significantly more cytotoxic than first mentioned material

1)test of fourfold table

Table 3. Results of scoring presence of bacteria after Brown and Brenn staining of tissue sections of teeth from 3 groups.

group / score	A monkey teeth sealed with Nimeticap	B dog teeth sealed with	C monkey teeth sealed with Cavit-W
-	5	14	17
+	11	10	5
Sample size	16	24	22

Table 4. Results of scoring inflammatory pulpal reaction to capping with Nimeticap and Cavit-W.

groups / score	A monkey teeth sealed with Nimeticap	B dog teeth sealed with Nimeticap	C monkey teeth sealed with Cavit-W
-	0	1	8
±	1	3	4
+	3	7	3
++	1	3	2
sample size	5	14	17

- no reaction
± scattered inflammatory cells
+ a focus of inflammatory cells
++ (nearly) generalized presence of inflammatory cells

Fourfold contingency table: A vs B p = 1.000
Mann and Whitney U-test: A + B vs C p = 0.008 (A+B>C)

It is known that filling materials can demonstrate different degrees of in vitro cytotoxicity when applied to a test sytem freshly mixed or after solification. Therefore, this variable was incorporated in the design of the present study. No evidence for a difference was found (Table 1).

ZnO-eugenol is known to be toxic when in direct contact with the pulp[5]). For this reason it was chosen as positive control in the in vitro experiment. It was hoped that when the sealants studied would demonstrate a lower cytotoxicity than ZnO-eugenol, this would predict more favourable results in vivo. In the present in vitro experiment, all filling materials investigated demonstrated a statistically significantly lower toxicity than ZnO-eugenol. Irrespective of the differences between the three formulas of Nimeticap, they appeared the most favourable materials when compared to all other sealants, used in this experiment. From pairs of the hydrophilic and of the hydrophobic filling materials Cavit and the three formulas of Nimeticap resp. showed the lowest cytotoxicity to human fibroblasts. According to the manufacturer the remanufactured olf formula might be more toxic than Nimeticap old formula because of slight differences in raw materials, whereas the new formula might be still less favourable because of both raw materials and a modification of specifications.
Consequently, they were chosen for the study of pulp tissue reactions. With regard to Nimeticap, the remanufactured old formula was used, demonstrating lower cytotoxicity than the new formula and being available to us.

In the in vivo experiment, 50% of the teeth appeared to be Brown and Brenn positive using Nimeticap. This might be due to the sealing procedure which was adopted for the cylindrical cavities of the present study where the least possible pressure was exerted to the materials to fill the cavity.
An investigation to overcome the problem of a high percentage of teeth containing microbes is going on.

For the study of tissue reaction only those teeth being Brown and Brenn negative were used.

Due to a shortage of deciduous monkey teeth, part of this study has been carried out using dog young-permanent teeth. Although differences in pulp tissue reactions between dogs and monkeys have been reported[6,7], the results of the present study do not tend to support these observations. However, in our case young-permanent dog teeth were compared with deciduous monkey teeth (Table 4). A difference in reaction between permanent and deciduous dentition might compensate a difference in animal species. Therefore, the results in dog and monkey teeth were pooled using the same sealant.

Contrary to the findings in vitro, Cavit appeared to provoke less severe tissue reaction than Nimeticap. An impetus for an explanation of this discrepancy between in vitro and in vivo results might be the observation of a precipitate under the pellets of Cavit in the in vitro experiment, contrary to Nimeticap where a precipitate was not observed. A detoxifying component, if any, might be trapped in the agar layer.

Compared with the results obtained by Mohammed, Van Huysen and Boyd[8], Seelig, Fowler and Tanchester[8] and Glass and Zander[9] for ZnO-eugenol, both Nimeticap and Cavit demonstrated more favourable pulp tissue reactions. This is in accordance with gross results in the in vitro experiment.

From the point of view of biocompatibility, Cavit-W appears to be the most favourable filling material from those investigated in this study. A study on its properties as a carrier for drugs is in progress.

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BACTERIA-TIGHT SEALING OF EXPOSED DOG PULPS

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Submitted

"It is important to note that the pulp can and does recover quite well from even severe insults, if these are of brief duration, but succumbs to low-grade insults, if these are persistent" (Massler, 1972). From this point of view a good seal between an exposed pulp and the oral environment is essential to eventual pulp healing, because it prevents the persisting insult of microleakage. The amount of leakage is partly dependant on its adhesion to both enamel and dentine and temperature changes (Going, Massler and Dute, 1960).

Both permanent and temporary restorative materials and materials used for endodontic temporary fillings were investigated for marginal leakage using techniques of penetration by dyes and bacteria, either in vitro or in vivo (Valcke and Kessler, 1978; Lamers, Simon and van Mullem, 1980; Martin, 1981). All tested materials demonstrated marginal leakage. Another experiment, both in vitro and in vivo, using a radioisotope technique revealed similar results on the leakage pattern under both conditions (McCurdy et al., 1974).

Factors such as smearlayer, varnishes and the adhesive strength of some cements to both enamel and dentine, were investigated in their relation to possible microleakage. Eventually, it was the acid-etch technique which promoted the adaptation of the resin to the tooth by altering the enamel, thereby enhancing considerabely the leakage resistance. In an in vitro study, with dye penetration, in which the temperature was changed twice daily and unfilled or gutta-percha filled cavities were covered with a UV polymerizing sealant, dye penetration appeared to have been

prevented for a period of 8 weeks. (The, van Mullem and Plasschaert, 1980).

The aim of this study was to investigate, to what degree a chemically and a UV polymerizing sealant could prevent marginal leakage of bacteria for the purpose of experimentation in animals.

MATERIALS AND METHODS

To investigate the sealant properties of Concise[†)] and Uvio-Bond[X)] under clinical conditions, exposures were made in 73 teeth of beagle dogs. The buccal surfaces of the teeth were cleaned with pumice and disinfected with 0.5% chlorhexidine digluconate in alcohol (Hibitane). The enamel surface peripheral to the planned cavity was treated with the etching solution belonging to the bonding to be used and according to the manufacturers' instructions. A glass bead sterilizer was used to disinfect the instruments. With a 1 mm Ø cylindrical burr cavities were drilled in the cervical third of the buccal surface of the crown. When the pulp was visible through the cavity floor exposure was performed by a dental explorer. After exposure the cavities were disinfected for 5 minutes with AF 1/10[+)], thoroughly dried with sterile paper points and sealed with

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[†)] 3M, St. Paul, Minn., U.S.A.

[X)] ESPE, Seefeld/Oberbay., W. Germany.

[+)] AF 1/10: 0.6 ml of an aqueous 35% formaldehyde solution (Merck, Darmstadt, Germany, analytical grade, catalog. no. 4003) and 2 ml 100% ethyl alcohol were added to 17.4 ml of water.

Cavit-W[X)]. The marginal enamel and the Cavit were covered with a layer of Concise in 25 teeth (experimental period 14 days) or with a layer of Uvio-Bond in 48 teeth (experimental periods 14 and 42 days, each 24 teeth). The Uvio-Bond was UV polymerized according to the manufacturers' instructions.

Each fortnight all teeth were checked for the presence of the Concise or Uvio-Bond layers.

After sacrifice, using a overdose of Nembutal, perfusion was performed, first with physiological saline solution then with a neutral 4% formaldehyde solution. Blocks of bone containing teeth in situ were dissected and postfixed in the same fixative, decalcified and embedded in Paraplast. Mesiodistal sections of the teeth, 7 µm thick, were Brown and Brenn stained. Evaluation of the sealing properties of Concise or Uvio-Bond was performed by scoring the Brown and Brenn stained sections for micro-organisms.

Statistical comparison was performed using an exact version of Fisher's test for fourfold tables. Testing was performed one-sidedly on the basis of the in vitro results (The, van Mullem and Plasschaert, 1982). The level of significance was fixed at 0.05.

RESULTS

In the 14-day group of 25 teeth which were covered with Concise 8 teeth appeared to have lost their layer of bonding, 6 of which demonstrated to be Brown and Brenn posi-

tive (28%). All other teeth (19) were Brown and Brenn negative.

In the 14-day group of 24 teeth which were covered with Uvio-Bond, 1 tooth lost its bonding and this tooth was Brown and Brenn positive (4.5%).

Statistical comparison of the Brown and Brenn scoring results of these groups revealed a p-value of 0.028.

All 24 teeth with the experimental period of 42 days retained their layer of Uvio-Bond and were Brown and Brenn negative.

DISCUSSION

From the point of view of biocompatibility Cavit-W was chosen in this investigation as the material to fill the cavity after exposure on the basis of an earlier study in which Cavit-W appeared to be a comparatively favourable cement for capping purposes (Wijnbergen, van Mullem and Wolters, 1982). However, Cavit appeared to permit microleakage of bacteria. After endodontic treatment, the access cavities were sealed with Cavit-W. Microleakage was considered to be responsible for the statistically significant increase with time (up to 42 days) in the number of teeth which were positive for micro-organisms (Lamers, Simon and van Mullem, 1980).

Covering Cavit fillings with an enamel adhesive bonding has been shown to prevent microleakage (Buonocore, Sheykholeslam and Glena, 1973; The, van Mullem and

Plasschaert, 1982). The in vitro investigation by The, van Mullem and Plasschaert (1982), in which dye penetration was prevented for a period of 8 weeks by covering unfilled or gutta-percha filled cavities with a UV polymerizing bonding (Estilux) is extended by the present in vivo experiment where penetration of bacteria was the parameter.
In this investigation, after an experimental period of 14 days, the 24 cavities sealed with Cavit and an Uvio-Bond layer covering the enamel and the Cavit demonstrated statistically significantly less microleakage of bacteria than the 25 cavities which were sealed with Cavit and Concise bonding. This result was supported by a 100% success rate after 42 days in 24 cavities which were sealed with Cavit-W and an Uvio-Bond layer.

In middle long term (6 weeks) animal experimentation a bacteria-tight seal of cavities can be achieved using Uvio-Bond to cover the (Cavit-W) filling material and the surrounding enamel.

SUMMARY

Penetration of bacteria past filling materials can interfere with the vitality of exposed pulps. In the present study, 73 dog's teeth were filled - after exposure - with Cavit-W and then sealed either with a chemically or a UV polymerizing bonding.

After 14 days a failure rate of 28% was demonstrated using the chemically polymerizing Concise and of 4.5% using the UV polymerizing Uvio-Bond. After 42 days the latter bon-

ding revealed a success rate of 100%.

To achieve a bacteria-tight seal of deep cavities for middle long term animal experimentation, Uvio-Bond can be used - after etching - to cover the filling material and the surrounding enamel.

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RELEASE OF AN ANTI-INFLAMMATORY DRUG FROM SOME DENTAL CEMENTS

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A dental restorative procedure should be considered to inflict a wound upon a tooth. Removal of infected dentin from a deep carious lesion can even result in a serious wound of the dental pulp: a so called pulp exposure. An exposure evokes an inflammatory reaction or is able to enhance an already existing one. This jeopardizes the survival of the pulp and may lead to extraction of the tooth.

Apart from the need of disinfection of the wound, the adminstration of an antiphlogistic to the pulp is supposed to be of great help to suppress the vascular phase of the inflammatory reaction and to prevent the development of a generalized inflammation, thus tending to the recovery of the pulp by an (indirect) promotion of a proliferative tissue reaction.

The period of action of such an anti-inflammatory drug after a single (pulse) administration to the wound - prior to filling of the cavity - was considered too short to be effective. Repeated systemic administration would exert an unnecessary influence on the body as a whole and would produce peak and valley concentrations at the site of action.
Therefore, its was decided to study administration from a controlled release system. Delivery over at least a period of several days can be expected from such systems. From the existing types of systems[1] the diffusion-controlled matrix system was chosen. Here the drug is homogeneously distributed over the carrying matrix and diffusion is the release rate determining factor.

As a first approach, dental cements were chosen as carrying matrices for the drug because they were easily available to us and, if effective in suppressing an inflammatory reaction, a cement containing drug would be cheaply to manufacture.

A material which is applied to cover the wound and the adjacent dentine, after an exposure, should be biocompatible and must provide sufficient strength to the cavity floor to enable the subsequent application of a filling material.

Since the dental cements chosen fulfil the requirements of biocompatibility[2] and strength, it is of interest to study the release of anti-inflammatory drugs from these materials.

The aim of this study was to investigate the in vitro release of the antiphlogistic Tantum from a number of commerially available dental cements, used as carrying matrices for the drug.

MATERIALS AND METHODS

Benzydamine-hydrochloride[x)] (Tantum^{R}; 1-benzyl-3-⌈3(dimethylamino)propoxy⌋-1H indazole) was chosen in the present study because of its ready solubility in water and alcohol[3] and its antiphlogistic potentials, more specifically its potential to reduce postoperative traumatic

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x) N.V. Organon, Oss, The Netherlands

swelling[4] which is desirable because of the confinement of the pulp within hard tissue.

Irrespective of their use in dental practice the following commercially available dental cements were selected on the basis of their respective water sorption characteristics:

1) Cavit-W[+)], a hydrophilic one-component cement containing ZnO with high water sorption characteristics. It even requires water for solidification. Widerman, Eames and Serene[5] reported an increase of initial weight of 9.6% after 3 hours immersion in distilled water at 37°C due to water sorption and loss of substances. The present authors observed a weight increase of 5% after 1 and 3 hours at 25°C.
2) Durelon[+)], a two-component polycarboxylate cement with moderate potential for water sorption.
3) Visiodispers[+)], a one-component artificial resin with low water affinity.
4) Ketac[+)], a two-component glass-ionomer cement with low water affinity
5) Nimeticap[+)], a two-component artificial resin. Its hydrophobic components provide very low water absorption characteristics to the set cement. It contains a quartz filler.

At the start of the experiment it seemed obvious to include Dycal - being frequently used in dental practice after pulp exposure - in this experiment. However, Dycal

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[+)] ESPE, Seefeld/Oberbayern, GFR.

containing Tantum could not be worked up into pellets because the mixture disintegrated.

Nimeticap, Ketac and Durelon are capsulated products with the powder and the liquid in separate compartments of the capsule. The Tantum powder was added to the powder of the cement. The capsule was shaken in a Silamat[a]) mixer, 30 times in a horizontal and 30 times in a vertical position. Then powder and liquid were mixed according to the manufacturer's instructions. Tantum powder was mixed by spatulation into the unset Visiodispers. For addition to Cavit, Tantum was dissolved in a minute amount of water. Then the solution was spatulated into the unset Cavit. The freshly mixed cements or the unset materials were pelletted by introduction into teflon rings (internal diameter 10 mm, 1,5 mm in height) laying on a glass slab. All cements, except Cavit-W, hardened without special precautions. The slabs carrying Cavit-W were placed in a moist atmosphere at 37°C. The final amount of the drug varied for each cement between 2,1 and 3,0 mg.

Pellets of each cement were introduced separately into 10 ml of two buffer solutions of different acidity. A citric acid-phosphate solution of pH 7,0 served as model for normal tissue and an acetic acid-sodium acetate solution of pH 4,8 served as approximation of inflamed tissue. Sample size was 3 - 6.

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[a]) Vivadent, Schaan, Lichtenstein.

The amount of Tantum which had leaked out of the pellets with time was measured using UV-spectrophotometry at λ_{max}= 306 mμ. A calibration curve was made using buffer solution with known concentrations of Tantum. If necessary, the experimental solutions were diluted in order to enable the determination of the amount of the antiphlogistic which had leaked out.
Mean percentages of released substance and their confidence intervals were calculated per group of pellets. Cumulative measurements were performed after the periods as shown in the Table and the Figures.
Control pellets of the cements - not containing Tantum - were studied to detect any substances which might be released and would interfere with the extinction at λ_{max} of Tantum.

RESULTS

In the controls no absorption at λ_{max} of Tantum was found. The short term release (up to 8 hours) of Tantum from Durelon and Cavit-W is given in Fig. 1 and the Table. The long term release from all cements studied (up to 7 days) is given in Fig. 2 and the Table.

DISCUSSION

As from the control cement pellets no substances leaked out the UV spectrum of which interferred with the maximal extinction of Tantum, the release percentage of the drug which were calculated on the basis of UV spectroscopy, can be compared mutually.

Fig. 1. Cumulative short term mean release percentages of Tantum from various dental cements.

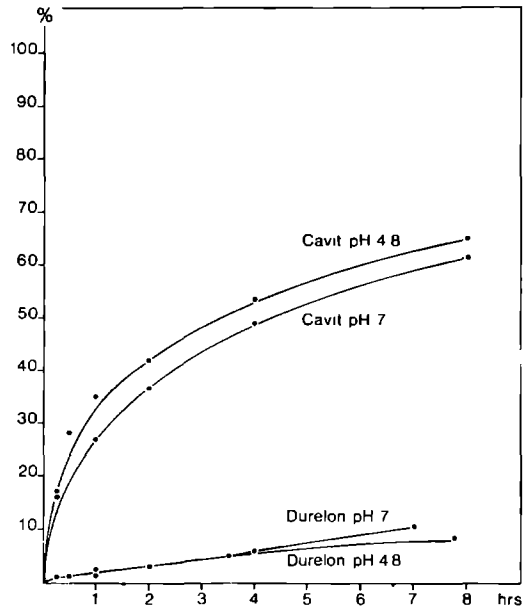


Table. Cumulative release of Tantum from pellets made of 5 dental cements.

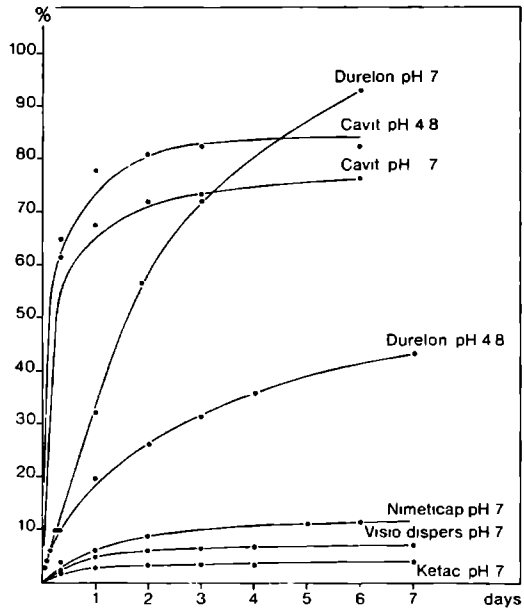
Mean release percentages and their confidence intervals.

	Cavit-W		Durelon	
	pH 4,8	pH 7,0	pH 4,8	pH 7,0
15 min.	17,3 ± 3,3	16,0 ± 1,0	-	0,9 ± 0,5
30 "	28,3 ± 7,3	-	1,3 ± 0,0	-
1 hour	35,0 ± 8,3	26,9 ± 1,4	2,5 ± 0,9	1,6 ± 0,5
2 hours	42,3 ± 5,7	36,7 ± 2,7	3,0 ± 0,0	3,1 ± 1,0
3½ "	-	-	5,0 ± 1,1	-
4 "	53,7 ± 4,3	48,7 ± 4,2	-	5,6 ± 1,6
7 "	-	-	-	10,5 ± 2,1
8 "	65,3 ± 9,7	61,6 ± 6,7	8,6 ± 1,6	-
1 day	77,7 ±12,7	67,9 ± 7,9	14,1 ± 3,9	32,4 ± 2,6
2 days	80,7 ±13,3	72,4 ± 9,1	17,3 ± 4,3	57,6 ± 0,4
3 "	82,3 ±16,7	73,6 ± 9,0	17,0 ± 4,2	72,0 ± 4,6
4 "	-	-	18,6 ± 9,6	-
5 "	-	-	-	-
6 "	82,3 ±16,7	76,4 ± 9,0	-	93,2 ± 9,0
7 "	-	-	26,3 ±11,5	-

- : No observations.

Ketac	Nimeticap		Visiodispers
pH 7,0	pH 4,8	pH 7,0	pH 7,0
0,4 ± 0,2	-	1,8 ± 0,2	0,9 ± 0,3
0,8 ± 0,3	-	-	1,5 ± 0,1
0,9 ± 0,3	2,3 ± 1,5	2,3 ± 0,2	2,1 ± 0,4
1,1 ± 0,5	2,9 ± 2,1	2,8 ± 0,4	2,4 ± 0,3
-	-	-	-
1,5 ± 0,6	3,9 ± 2,4	3,3 ± 0,4	3,1 ± 0,2
-	-	-	-
2,1 ± 0,7	5,3 ± 3,0	4,3 ± 1,1	3,8 ± 0,4
2,6 ± 0,7	7,0 ± 3,3	6,3 ± 1,9	4,8 ± 0,4
3,0 ± 0,7	8,1 ± 2,7	9,0 ± 2,2	5,6 ± 0,6
3,3 ± 0,7	8,8 ± 3,3	-	5,9 ± 0,5
3,5 ± 0,8	9,2 ± 3,6	-	6,0 ± 0,6
-	-	10,9 ± 2,1	-
-	-	11,5 ± 1,8	-
4,0 ± 0,4	-	-	6,2 ± 0,7

Fig. 2. Cumulative long term release percentages of Tantum from various dental cements.



Release which is directly proportional to time (zero-order release), was considered desirable over a period of at least 7 days after exposure to decrease an inflammatory tissue reaction. However, such zero-order release was not found.

All low water sorption cements (Nimeticap, Visiodispers and Ketac) demonstrated a low drug release only which mainly took place during the first day (Fig. 2). This indicates that only those Tantum particles located at or very close to the surface of the pellet, were dissolved.

Using cements with higher water sorption characteristics (Durelon and Cavit) substantial amounts of antiphlogisticum were released during the first day, with the exception of Durelon at pH 4,8. Here a slow release was observed following a 20% release after the first day (Fig. 2). The release from Durelon at pH 7,0 suggested to be of zero-order over the first two days (Fig. 2). After that period of time the percentage released material amounted to 60%. This was followed by a slower release to 90% after 6 days (Fig. 2).

Apparently, the polycarboxylate cement is the only material studied the release of which is significantly pH dependant. Probably the explanation for this phenomenon should be found in the fact that the solubility of the cement itself is pH controlled. This is not the case with the other materials studied.
Cavit-W delivered the drug for the major part (60-65%) during the first 8 hours (Fig. 1) after which some release took place up to 2 days at which time 70-80% of the initial amount had leaked out (Fig. 2). This pattern of re-

lease can be understood by the pronounced water absorption-desorption characteristics of this material.

The described results suggest that for studying biological effects of Tantum release, Cavit-W should be used as matrix in the case short term release (2 days) is envisaged and Durelon may be used in the case the effects of long-term release (5-7 days) is to be studied.

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SYNOPSIS

Controlled release of the antiphlogisticum Tantum was studied from a number of commercially available dental cements used as matrices.
Low water sorption cements released only minor amounts. Cavit released the major part (75%) within the first two days. The release from Durelon was pH dependant: at pH 4,8 20% was released after one day, at pH 7,0 55% after 2 days, which in both cases was followed by a slower release.

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EXPERIMENTALLY INDUCED PULPITIS BY INTENTIONAL INFECTION AFTER EXPOSURE

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In the clinical situation, exposure of the pulp can be the result of removing carious dentine and under such circumstances usually a pulpitis exists. Experiments on the process of pulpal healing should simulate clinical conditions as closely as possible. For such studies, a method for induction of an inflammatory reaction of a moderate degree in exposed but originally healthy teeth is required. A period of 2 days for the induction of a pulpitis is convenient in a protocol pertaining to studies on the effects of drugs.

Mjör and Tronstad (1972) and Lervik and Mjör (1977) studied ways of obtaining a standardized pulpitis in initially healthy monkey teeth. Cavities were prepared with the floor in the inner third of the dentine. Cumulative to this trauma they induced inflammatory reactions of the pulp in one of the following ways:

1) soft carious human dentine was placed on the floor of the cavities (the cavities were filled with amalgam)
2) the cavities were filled with gutta-percha only, or
3) the cavities were left open.

Histological evaluation revealed that the reactions to open cavities varied too widely to be compatible with the aim of the investigation (Mjör and Tronstad, 1972). However, a standardized and reproducible pulpitis could be obtained when human carious dentine was used. An experimental period of 2-5 days was to be preferred to 8 days for inducing a severe inflammation. In the method using gutta-percha, a period of 2 - 5 days gave rise to a slight reaction of the pulp, which was less severe than that using

human carious dentine. After a period of 8 days the reaction was moderate and after 10 days severe (Lervik and Mjör, 1977).

Infection with Strep. faecalis of the exposure site has been performed by Iserman and Kaminski (1979). The 5 beagle dog teeth appeared to be positive after reentering the teeth and culturing after 3 days. However, in their study, the effect of infection was not studied histologically after that interval.

When sticking to an induction period of 2 days, an average slight reaction can be too slight and an average severe reaction too strong to observe the effects of drugs in various concentrations. Therefore, the purpose of this study was to investigate whether a Strep. faecalis infection of exposed pulps, followed by a sealing with gutta-percha would provoke an average moderate inflammatory reaction after 2 days.

MATERIALS AND METHODS

Exposures were made in 16 caries-free permanent teeth of beagle dogs. The operating field was disinfected with Hibitane (0.5% chlorhexidine digluconate in alcohol) and a glass bead sterilizer was used for disinfection of the instruments to be sure that only controlled infection took place. After exposure of the pulp the cavities were filled by syringe with a freshly prepared aqueous suspension of 10^6 Strep. faecalis per ml. After 5 minutes the cavities were dried with sterile paper points and sealed with gutta-percha point sections, the length of which was shorter

than the depth of the cavities. The enamel around the cavities was etched and covered with a layer of Uvio-Bond[X]. The teeth were extracted after an experimental period of 2 days and fixed in neutral 4% formaldehyde solution. After embedding in Paraplast the inflammatory reaction was studied in 7 µm thick haematoxylin-eosin stained sections. The following 4-point scale was used for scoring (weighting factors in brackets):

- : no reaction (0),
± : slight reaction (1): scattered inflammatory cells,
+ : moderate reaction (2): a focus or band of inflammatory cells, and
++ : severe reaction (3): abscess formation or a reaction demonstrating strongly degenerative tissue changes.

Pulpal tissue at a distance from the site where the cut dentinal tubules open into the pulp served as control material.

RESULTS

The results of scoring inflammatory reactions were: slight in 8 teeth, moderate in 4 teeth and severe in 4 teeth. None of the pulps revealing severe reactions demonstrated abscess formation. All showed localized degenerative tissue changes. The mean tissue reaction was 1.75, a moderate reaction being 2 arbitrary units.

X) ESPE, Seefeld, Oberbayern, GFR.

DISCUSSION

The histological results obtained in this study, in which the exposed pulp was infected with Strep. faecalis and was left for 2 days beneath a filling of gutta-percha point sections, indicated that the provoked pulpitis was of an approximately moderate degree. There were no teeth demonstrating no reaction. This is of importance when studying the recovery of pulp tissue from traumata in which complete healing is aimed at. A moderate degree was also obtained by Mjör and Tronstad (1972) and Lervik and Mjör (1977) but after 8 days, using gutta-percha fillings in deep cavities (i.e. up to inner third of the dentine). From the differences between their study and the present one (deep cavities versus exposure, no infection versus infection) the infection with Strep. faecalis in this study is considered the major factor for the earlier attainment of a moderate inflammatory reaction.

SUMMARY

A 5-minute application of an aqueous 10^6/ml suspension of Strep. faecalis to exposed pulps in experimental animals appeared to induced an approximately moderate inflammatory reaction after 2 days. This method is considered to be suitable for studies on healing of inflamed pulpal tissue.

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HISTOLOGICAL STUDY OF THE EFFECT OF CONTROLLED RELEASE OF AN ANTIPHLOGISTICUM ON EXPOSED INFLAMED DOG PULPS

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After the mechanical exposure of a pulp an acute inflammation occurs at the wound surface. This inflammation can remain or change into a chronic inflammation. The pulp can even become necrotic. In which direction the inflammation will develop depends on the presence or absence of multiple and complex phenomena within the pulp, such as the amount of destroyed tissue, an infection, hemorrhages, obstruction of the blood supply which is essential to tissue repair, and factors that determine the general health of the patient. Moreover, the wound has to be fenced off from the saliva, because marginal leakage has to be regarded as a threat to the survival of the pulp.

Seltzer[1] (1975) gives a summary of the materials used for capping wounded pulp tissues and although some of them give relatively efficacious results, none of them is absolutely satisfying.
So the search for methods and materials for capping the wounded pulp tissue continues (Patterson[2], 1976;, Haskell, Stanley, Chellemi, et al.[3], 1978; Pereira, Bramante, Berbert et al.[4], 1980; Pereira and Stanley[5], 1981; Dick and Carmichael[6], 1980; Eleazer, Bolanos and Sinai, 1981).

The ideal material for use in contact with an exposed pulp has not yet been found, and there seems to be a tendency to accept a dead pulpodentinal organ, instead of aiming at a vital pulp. In other places of the body, a connective tissue wound results in an inflammatory reaction, and in most cases is followed by complete repair with or without the aid of medicaments. Why should this not be possible with pulp tissue, being essentially a connective tissue

although it is confined by hard tissue?

As success or failure of a pulp capping procedure depends upon the absence or presence of pulpal inflammation rather than upon the formation of a calcified bridge, the purpose of this investigation was to study whether controlled release of the antiphlogistic Tantum from a dental cement could act to reduce the inflammatory reaction in a wounded and inflamed pulp.

Pulse administration of a high amount of Tantum might exert toxic effects and repeated administrations would require the re-opening of the cavity. For both of these reasons a controlled release system is preferred.

The pattern of release of Tantum was investigated in vitro by Wijnbergen, van Mullem and de Wijn[8] (1982 a) from two dental materials: Cavit and Durelon. Cavit demonstrated a rather favourable biocompatibility after exposure (Wijnbergen, van Mullem and Wolters[9], 1982 b) and Durelon, although initially irritating, was well tolerated by the pulp (McWalter, El-Kafrawy and Mitchell[10], 1973; El-Kafrawy, Dickey and Mitchell[11], 1974). Cavit demonstrated a quick release during the first 48 hours followed by a slight release up to 7 days. Durelon demonstrated a more gradual release over a 1-week period.

To demonstrate the antiphlogistic action on bacterially inflamed pulps, a method of creating a controlled inflammatory reaction was required. An approximately moderate reaction could be obtained after 2 days by infecting the pulp tissue with Strep. feacalis (Wijnbergen and van Mullem[12], 1982 c).

Prior to the application of the antiphlogistic, the wound was disinfected with AF 1/10, which demonstrated that it possessed antibacterial power (Wijnbergen and van Mullem [13], 1982 d), avoided circulatory stasis (van Mullem and Wijnbergen[14], 1982 e) and otherwise added no noxious effect to that of the exposure[13]. An adequate supply of blood is of vital importance for the response of the pulp to injury.

MATERIALS AND METHODS

Ninety caries-free teeth of four 9-month old Beagle dogs were used in this study. The dogs were anaesthetized using Nembutal and the operation field was disinfected with Hibitane (o.5% chlorhexidine digluconate in alcohol). A glass bead sterilizer was used to disinfect the instruments to be sure that only controlled infection took place.

With a 1 mm Ø cylindrical bur (using low speed and air-cooling) cavities were drilled in the buccal surfaces of the teeth. When the pulp was visible through the cavity floor the pulp was exposed by means of an explorer. All cavities were infected by the introduction of a freshly prepared aqueous 10^6/ml suspension of Strep. faecalis to induce a controlled inflammatory tissue reaction of bacterial origin (Wijnbergen and van Mullem[12], 1982 c). After 5 minutes the cavities were dried with sterile paper points and sealed with gutta-percha point sections. After etching of the surface of the enamel which surrounds the cavity, a layer of Uvio-Bond was applied.

The sealants were removed after 2 days and the cavities were disinfected during 5 minutes with AF 1/10[x)] and dried with sterile paper points.

Then the cavities were sealed with the dental cement carcier containing the antiphlogistic or not. When Cavit-W served as carrier, Tantum was dissolved in water which was then spatulated into Cavit.
Where Durelon served as carrier, Tantum powder was mixed with the powder of Durelon in the capsule. This powder mixture was then mixed with the Durelon liquid in accordance with the manufacturers' instructions. With both carriers the end concentration of the drug was 1%W/W. After sealing, the cavities and surrounding enamel of all teeth were covered, after etching, with Uvio-Bond to provide a micro-organism tight seal (Wijnbergen and van Mullem[15], 1982 f).

The experiment comprised 4 groups of teeth - cavities sealed with Cavit, Cavit containing Tantum, Durelon, or Durelon containing Tantum - for each experimental period: 2 and 7 days.

At the end of the experimental period the dogs were anaesthetized and perfused, first with physiological saline solution, then with a neutral 4% formaldehyde solution. The parts of the dog's jaws that contained the teeth

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x) AF 1/10: 0.6 ml of an aqueous 35% formaldehyde solution (Merck, Darmstadt, Germany, analytical grade, catalog no. 4003) and 2 ml 100% ethyl alcohol were added to 17.4 ml of water.

in situ were removed and immersed in the same fixative. After dissection the teeth were decalcified and embedded in Paraplast. Seven µm thick bucco-lingual sections in which the cavities appeared longitudinally, were haematoxylin-eosin or Brown and Brenn stained.

The presence or absence of bacteria was scored using the Brown and Brenn stained sections. For evaluation of the pulp tissue reaction, only those teeth which were Brown and Brenn negative were used, to exclude reaction of bacterial origin. In the histopathological analysis, the inflammatory reaction and - separately - necrosis were scored at the wound surface and at a site 2000 µm in apical direction of the wound surface.

The tissue reactions were scored according to the following three - and four-point scales which were used for statistical comparison.
Weighting factors are given in brackets and are used for calculation of mean tissue reactions.
For inflammatory reaction a four-point scale was used:

- (0) : no reaction
± (1) : scattered inflammatory cells
+ (2) : more scattered inflammatory cells to a focus
++ (3) : abscess formation

Necrosis was scored according to a three-point scale:

- (0) : no reaction
± (1) : autolysis of a few cells
+ (2) : autolysis of more areas of cells, sometimes showing eosinophilic staining and containing small vacuoles.

Mean tissue reactions - for inflammatory reaction and separately for necrosis - were calculated, combined for the scores at the wound surface and at the site 2000 µm in apical direction, and were plotted graphically.

Statistical comparisons of mutually dependent scoring results which were obtained pairwise from each tooth, were performed using the signed-rank test. Independent series of scoring results, originating from different groups of teeth and obviously those from the wound surface separately from those of 2000 µm lower, were tested by the Mann and Whitney U-test (Wilcoxon-test) or, in case this was not allowed, by an exact version of Fisher's test for fourfold tables.
Tests were performed one-sidedly on the basis of in vitro results where Durelon was more toxic than Cavit-W (Wijnbergen and van Mullem[9], 1982 b) or because only decrease of the inflammatory reaction was the expected action of the antiphlogistic. With regard to necrosis, an increase after 2 days and a decrease after 7 days was of interest. As level of significance $\alpha = 0.05$ was chosen, whereas $0.10 > p > 0.05$ was considered nearly significant.

RESULTS

Of the 90 exposed teeth, 78 were Brown and Brenn negative and thus were valuable for histological evaluation. The distribution of the numbers of these teeth over the experimental groups and the frequency distribution of the scores per group are given in Tables 1 and 2.

Table 1. Results of scoring inflammatory reaction and necrosis after capping with Cavit or Cavit containing Tantum after 2 and 7 days.

Exp. period	Score	Teeth sealed with Cavit			
		inflammatory reaction at		necrosis at	
		wound surface	2000 µm	wound surface	2000 µm
2 days	-	3	7	2	6
	±	6	5	9	8
	+	7	5	6	3
	++	1	0	0	0
		17	17	17	17
7 days	-	3	6	0	2
	±	6	3	4	3
	+	0	0	2	2
	++	0	0	3	2
		9	9	9	9

Teeth sealed with Cavit containing Tantum			
inflammatory reaction at		necrosis at	
wound surface	2000 µm	wound surface	2000 µm
4	6	1	2
6	2	3	7
1	3	6	0
0	0	1	2
11	11	11	11
4	7	0	5
4	2	7	3
2	1	0	0
0	0	3	2
10	10	10	10

Table 2. Results of scoring inflammatory reaction and necrosis to capping with Durelon and Durelon with Tantum after 2 and 7 days.

Exp. period	Score	Teeth sealed with Durelon			
		inflammatory reaction at wound 2000 µm surface		necrosis at wound 2000 µm surface	
2 days	-	1	3	0	3
	±	4	3	3	4
	+	3	2	5	1
	++	1	1	1	1
		9	9	9	9
7 days	-	2	4	1	3
	±	5	6	3	5
	+	3	0	6	2
	++	0	0	0	0
		10	10	10	10

Teeth sealed with Durelon + Tantum			
inflammatory reaction at wound surface	2000 µm	necrosis at wound surface	2000 µm
0	3	0	0
5	0	0	3
1	2	5	1
0	1	1	2
6	6	6	6
3	4	0	2
2	2	4	3
1	0	1	0
0	0	1	1
6	6	6	6

The p-value of the statistical comparison of the scoring results obtained at the wound surface versus those at the site 2000 µm apically of the wound surface are given in Table 3.

The scoring results for Cavit were compared with those for Durelon (both without drugs). Regarding inflammatory reaction the p-values for the wound surface and 2000 µm apically, resp., were: after 2 days 0.425 and 0.323 and after 7 days 0.124 and 0.242, resp. Similar comparisons regarding necrosis revealed p-values of 0.133, 0.375, 0.230 and 0.152, resp.

Comparison of the results obtained with Cavit or Durelon, with versus without Tantum, revealed the p-values given in Tables 4 and 5, resp.

The mean tissue reactions after 2 and 7 days to Cavit or Durelon, each with or without Tantum, were calculated and - for the sake of clarity, combined for wound surface and 2000 µm apically - plotted graphically in Fig. 1.

In the group of 28 teeth of which histological processing started two days after sealing with Cavit or Cavit containing Tantum, principally neutrophilic leucocytes were seen at the boundary of the pulp tissue and the sealing material. In the group of 19 teeth which were fixed 7 days after sealing, a majority of macrophages was observed in the inflamed tissue.

Table 3. p-Values of comparisons of scoring results obtained at the wound surface and at a site 2000 µm in apical direction.

Groups	Cavit		Groups	Durelon	
	inflammatory reaction	necrosis		inflammatory reaction	necrosis
C2	0.064↓	0.021↓	D2	0.334	0.039↓
C7	0.149	0.121	D7	0.045↓	0.192
CT2	0.452	0.059↓	DT2	0.456	0.212
CT7	0.036↓	0.024↓	DT7	0.174	0.074↓

C: Cavit, CT: Cavit containing Tantum: D: Durelon, DT: Durelon containing Tantum, after 2 or 7 days,

↓: tissue reaction at the site 2000 µm in apical direction statistically (nearly) significantly less severe than at wound surface.

Table 4. p-Values of tests on observations concerning Cavit and Cavit containing Tantum.

			wound surface	2000 µm apically
Inflammatory reaction				
1. C2	vs	C7	0.020↓[1]	0.059↓[1]
2. C2	vs	CT2	0.027↓[1]	0.316 [1]
3. C7	vs	CT7	0.263 [2]	0.526 [2]
4. CT2	vs	CT7	0.452 [1]	0.331 [2]
Necrosis				
5. C2	vs	C7	0.045 [1]	0.076 [1]
6. C2	vs	CT2	0.969 [2]	0.710 [2]
7. C7	vs	CT7	0.255 [1]	0.134 [1]
8. CT2	vs	CT7	0.337 [1]	0.161 [1]

Legends: see Table 3 and:

↓: tissue reaction in second mentioned group statistically (nearly) significantly less severe than in first mentioned group.

[1]: Mann and Whitney U-test

[2]: fourfold table - and vs + and ++

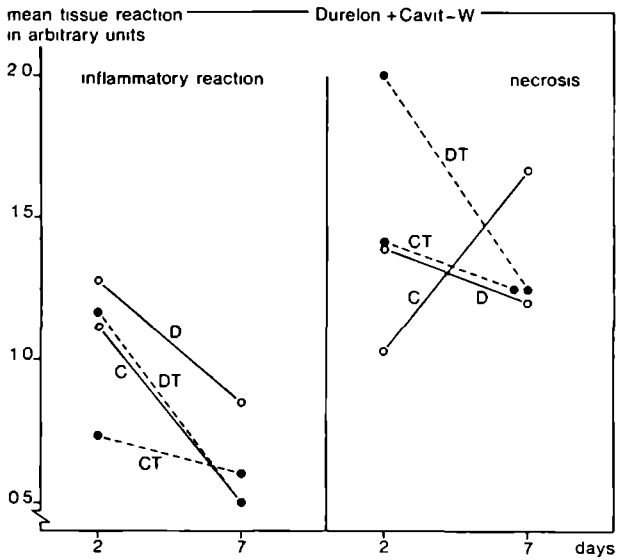
Table 5. p-Values of tests on observations concerning Durelon and Durelon containing Tantum.

			wound surface	2000 µm apically
Inflammatory reaction				
1. D2	vs	D7	0.215 [2)]	0.154 [1)]
2. D2	vs	DT2	0.294 [2)]	0.476 [1)]
3. D7	vs	DT7	0.149 [1)]	0.174 [1)]
4. DT2	vs	DT7	0.091↓ [2)]	0.091↓ [2)]
Necrosis				
5. D2	vs	D7	0.780 [2)]	0.500 [1)]
6. D2	vs	DT2	0.185 [2)]	0.052↑ [1)]
7. D7	vs	DT7	0.382 [1)]	0.500 [2)]
8. DT2	vs	DT7	0.049↓ [1)]	0.084↓ [1)]

Legends: see Table 4 and

↑: tissue reaction in second mentioned group statistically (nearly) significantly more severe than in first mentioned group.

Fig. 1. Mean tissue reactions - the scores for the wound surface were combined with those at 2000 µm apically - for Cavit and Durelon with or without Tantum after both experimental periods.



DISCUSSION

The study of the influence of an antiphlogistic drug on the tissue reaction in the dental pulp after exposure was performed on bacterially infected pulps. This was done to simulate clinical conditions as closely as possible. Obviously, disinfection was carried out before the drug was applied.

The fact that a large number of teeth (12) out of the total of 90 demonstrated to be Brown and Brenn positive, cannot be explained by microleakage of the sealant which was used after disinfection. The Cavit or the Durelon was covered with a layer of Uvio-Bond. This method of sealing was found to be bacteria-tight (Wijnbergen and van Mullem [15], 1982 f). Beside the possibility of an occasional contamination in the short period between drying with sterile paper points and sealing, the short period of time - 5 minutes - during which the disinfectant (AF 1/10) was used might be suspected.
This is seemingly in contradiction to the results of the study where the disinfectant was tested for effectiveness (Wijnbergen and van Mullem[12], 1982 c). Here infection of the wounded pulp was immediately followed by a 5-minute' disinfection, thus simulating the clinical phenomenon of contamination. However, in the present experiment where a pulpitis was induced, the 5-minute infection was followed by 2 days during which bacteria may have become located at sites (e.g. far inside dentinal tubuli) which were not easily reached by the disinfectant during its 5 minutes of action.

When in each experimental group the scoring results for

inflammation or necrosis at the wound surface and at the site 2000 µm in apical direction were compared by means of signed-rank tests, 5 out of the 16 p-values indicated significance and 3 near significance (Table 3).
In all these cases the direction of (near) significance was similar: a less severe tissue reaction at 2000 µm than at the wound surface, which indicated that the pulpal tissue over a length of 2000 µm was involved in the tissue reaction to a lesser degree in these (near) significant cases than in the non-significant cases.

In this investigation it was assumed that Durelon would provoke more severe tissue reaction than Cavit. All tests performed to study differences were not even nearly significant. Therefore, there is no reason to believe that Durelon is more toxic to the exposed pulp than Cavit-W.

Cavit-W (Fig. 1).

The inflammatory reaction to the operation trauma and the capping with Cavit appeared to be significantly more severe at the wound surface, and nearly significantly so at a site 2000 µm apically, after 2 days than after 7 days (Table 4, line 1). This reduction of the inflammation indicated that the pulp - under the conditions in this study - can at least partly recover without the aid of an antiphlogistic drug.

When Tantum was added to the Cavit the inflammatory reaction at the wound surface after 2 days was significantly less than with Cavit only (Table 4, line 2). Tantum appeared to have exerted a favourable influence during the first two days after application.

After this initial suppression of the inflammatory reaction no further decrease appeared to take place (Table 4, line 4).Comparison of the results obtained after 7 days with and without Tantum (Table 4, line 3) revealed no statistical significance. The end result - after 7 days in this study - appeared to be similar for Cavit and Cavit containing Tantum.

These results demonstrated similarity with those obtained when the release of Tantum from Cavit was studied in vitro (Wijnbergen, van Mullem and de Wijn[8], 1982 a). Approximately 75% of the Tantum had leaked out after 2 days after which only minor amounts were released.
This suggested that the amount released during the first two days was effective, whereas the small subsequent amount was not.

When Cavit without drug was used, necrosis appeared to be more severe at the wound surface and at 2000 µm apically after 7 days than after 2 days (Table 4, line 5).
When tested one-sidedly for reduction in necrosis with lapse of time, these p-values methodologically could not be considered statistically (nearly) significant, because necrosis was more severe after 7 days than after 2 days. However, the direction of the line (C in Fig. 1) which was in contrast to that of all other lines and its steep slope, were convincing evidence that the difference between 2 and 7 days was significant. This increase in necrosis might be seen as a threat to the vitality of the pulps which were intentionally inflamed in this study. However, longer experimental periods are needed to answer this question.

For Cavit containing Tantum, no decrease in necrosis was found after 2 and 7 days (Table 4, lines 6 and 7). When the results after 7 days were compared with those after 2 days (Table 4, line 8) no significant decrease was revealed. However, for Cavit containing Tantum, the site 2000 µm apically was significantly less involved in the tissue reaction than at the wound surface after 7 days (Table 3). This is in contrast to what is seen after 7 days for Cavit. This can be taken as an indication that Tantum slightly inhibited necrosis during the period between 2 and 7 days.
Consequently, the use of Tantum seemed to exert a clearly favourable influence on the inflammatory reaction and but a slightly favourable influence on necrosis.

Durelon (Fig. 2).

Reduction of the inflammatory reaction, using Durelon without drug, could not be ascertained (Table 5, line 1). Influence of the addition of Tantum to Durelon was not found after 2 and 7 days (Table 5, line 2 and 3). But nearly significant reduction of the inflammatory reaction was found over the period from 2 - 7 days, both at the wound and 2000 µm apically (Table 5, line 4).

Studying controlled release of Tantum from Durelon, Wijnbergen, van Mullem and de Wijn[8],(1982 a) found that after release of approximately 25-55% of the drug initially added to the Durelon during the first two days - which is distinctly lower than from Cavit - the release continued, although more slowly over the period from 2 - 7 days.

The above mentioned slight reduction of the inflammatory reaction over the period from 2 - 7 days can be explained as a result of the continued release on top of an, initially, lower amount than with Cavit.

Necrosis was found to be increased after 2 days when Tantum was added to Durelon (Table 5, line 6). However, a significant reduction was noted at the wound surface and a nearly significant reduction 2000 µm apically after the period from 2 - 7 days (Table 5, line 8).
This reduction - after an initial enhancement of the necrosis by Tantum contained in the Durelon carrier - can also be due to the continued release of the drug from Durelon.

Consequently, Tantum appeared to exert a (nearly) significant favourable influence on inflammatory reaction and necrosis when released from Durelon.

This study revealed evidence that the system of controlled release of an antiphlogisticum from a biomaterial as matrix (carrier), when applied to an exposed inflamed pulp, is effective, but much experimental work has to be performed to optimalize this effect.

SUMMARY

The effect of controlled release of the antiphlogistic Tantum, from two carrier, Cavit-W and Durelon, on inflammatory and necrotic tissue changes was investigated on intentionally inflamed pulps of dogs.
Statistical tests to detect differences in biocompatibili-

ty between Cavit and Durelon, both without drug, led to the conclusion of a similar biocompatibility as determined after 2 and 7 days.
Tantum appeared to reduce necrosis during the period from 2 - 7 days slightly with Cavit as carrier and substantially with Durelon as carrier. However, in the latter case necrosis was enhanced after 2 days.
During the same period inflammatory reaction was decreased nearly significantly by Durelon containing Tantum. It was decreased significantly after 2 days with Cavit as carrier. Controlled release of Tantum from Cavit or Durelon which was placed on an exposed inflamed pulp was effective in reducing inflammatory reaction and necrosis.

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CURRICULUM VITAE

The author of this thesis was born in Rotterdam in 1931. She attended the Gymnasium Erasmianum, from which she obtained her β-diploma in 1950. She studied dentistry at the University of Utrecht, and graduated in 1957. Subsequently, she worked for ten years with the School Dental Service at IJsselmonde, Hoekse Waard and Arnhem.

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Since 1976, after a reorganization of the Faculty, she is staff member of the Department of Pedodontics (Head: Prof. A.J. van Amerongen).

She is married and has two children.

STELLINGEN

1. Door de conceptie van controlled drug release - waarbij therapeutische doses van een desinfectans en/of een niet-corticoid antiphlogisticum over langere perioden aan de pulpa worden afgegeven - in te voeren in ons denken over de behandeling van de geëxponeerde, geïnfecteerde pulpa, staat de directe pulpa-overkapping opnieuw open voor onderzoek.

2. De tijd is aangebroken om het verrichten van onderzoek over mogelijkheden van toediening van een agens ter bevordering van genezing van de geëxponeerde, geïnfecteerde pulpa te verkiezen boven het doen van onderzoek over agentia, waarvan vaststaat dat zij avitaliteit van de pulpa tot gevolg hebben.

3. Het kleine volume van de pulpa suggereert ten onrechte een evenredig klein vermogen tot genezing.

4. De directe overkapping van een geëxponeerde pulpa kan reeds succesvol genoemd worden, als de pulpa vrij is van ontstekingsverschijnselen.

5. Vorming van een hardweefsel brug na exponatie van de pulpa moet als een pathologische verandering gezien worden en is derhalve ongewenst.

6. Wanneer het woordgebruik in de orale histologie - waar de termen secundair, tertiair, reparatief en irritatie dentine door elkaar gebruikt worden - beperkt zou worden tot het gebruik van de term irritatie dentine, zou veel aan duidelijkheid gewonnen worden.

7. "De même qu'une inflammation quelconque du corps doit être soignée, qu'on ne coupe pas une jambe ou un doigt pour un panaris, ou pour une plaie, de même doit-on faire de la chirurgie conservatrice pour les pulpes malades ou pour leur débris".

   Camille Renard, Dr. méd., Professeur à la Policlinique de l'Ecole dentaire de Genève (1881-1910).

8. Ook al zijn niet alle bacteriën door een desinfectans gedood, dan nog kan vaak van een effectieve desinfectie van de pulpa gesproken worden.

9. Tot het vakgebied Kindertandheelkunde dient ook gerekend te worden de zogenaamde preventieve Orthodontie.
Gezien het feit dat er geen duidelijke scheidingslijn te trekken is tussen preventieve en interceptieve orthodontische maatregelen houdt dit in dat de Kindertandheelkunde en de Orthodontie geïntegreerd onderwezen dienen te worden.

10. Gezien de toename van orthodontische behandelingen door algemeen practici zijn P.A.O. cursussen op dit gebied dringend gewenst.
Tevens dient een herbezinning plaats te vinden op het gewenste aantal orthodontisten in Nederland.

11. Een verplicht stagejaar na het afstuderen is, voor tandartsen, niet alleen wenselijk, maar binnenkort ook noodzakelijk indien het subfacultaire patientenbestand niet spoedig uitgebreid en minder eenzijdig samengesteld wordt.

12. Het verzet van de Nederlandse Maatschappij tot Bevordering der Tandheelkunde tegen een uitbreiding van het aantal hulpkrachten op basis van de huidige prognoses met betrekking tot de tandartsendichtheid in Nederland getuigt van weinig visie.

13. De ontwikkeling van de Sony Walkman II kan vooral beschouwd worden als een zegen voor de familie van de muziekliefhebber.

M.G.J. Wijnbergen - Buijen van Weelderen

24 juni, 1982